VOLUME 44 | ISSUE 2 | YEAR 2020
ISSN 1300-0128 • E-ISSN 1303-6181

TURKISH JOURNAL OF VETERINARY & ANIMAL SCIENCES

http://journals.tubitak.gov.tr/veterinary/

Published by the Scientific and Technological Research Council of Turkey

TÜBİTAK

KORESPONDENSI

Nama Jurnal : Turkish Journal of Veterinary and Animal Sciences

Judul Artikel : Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)

No.	Proses	Waktu
1.	Submit manuskrip	24 Mei 2019
2.	Under review oleh editor jurnal	9 Juli 2019
3.	Revisi manuskrip oleh editor jurnal	9 Juli 2019
4.	Proof read manuskrip	23 Juli 2019
5.	Re-submit revisi manuskrip	1 Agustus 2019
6.	Under review oleh reviewer jurnal	1 Agustus 2019
7.	Revisi dari reviewer jurnal	30 September 2019
8.	Reminder dari editor jurnal	20 Oktober 2019
9.	Revisi manuskrip dan re-submit	4 November 2019
10.	Reminder dari editor jurnal	24 November 2019
11.	Revisi manuskrip dari re-submit	24 November 2019
12.	Revisi manuskrip oleh editor jurnal	2 Desember 2019
13.	Reminder dari editor jurnal	3 Desember 2019
14.	Revisi manuskrip dan re-submit	3 Desember 2019
15.	Accepted artikel pada jurnal	28 Januari 2020
16.	Publish artikel di jurnal (online)	6 Mei 2020

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Jumat, 24 Mei 2019 21.17 GMT+7

Dear AKHMAD MUKTI,

Your manuscript has been received and is currently being processed.

We thank you for your interest in our journal.

Yours sincerely,

Manuscript Title: Growth performance, survival, flesh percentage, and proximate composition of mono- and mixed-sex-cultured triploid and diploid Nile tilapia (Oreochromis niloticus)
Manuscript Code Number: VET-1905-79

YÜCEL UYAR
Journal Administrator

Note: For the manuscripts submitted via our online manuscript submission system, please use the following link:

http://online.journals.tubitak.gov.tr

Growth performance, survival, flesh percentage, and proximate composition of mono- and mixed-sex-cultured triploid and diploid Nile tilapia (*Oreochromis niloticus*)

Akhmad Taufiq MUKTI[1*], Odang CARMAN[2], ALIMUDDIN[2], Muhammad ZAIRIN Jr.[2], Muhammad Agus SUPRAYUDI[2]

[1] Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115, Indonesia

[2] Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB), Bogor 16680, Indonesia

Corresponding Author: atm_mlg@yahoo.com

Abstract: The aims of this study were compared the growth performance, survival, flesh percentage and proximate composition of mono- and mixed-sex-cultured triploid and diploid Nile tilapia fish. The triploid population was produced by heat shock treatment at 41°C for 4 minutes, 4 minutes after fertilization. Before sexing, fish were reared in aquaria at the density of 50 fish per aquarium for 2 months. After sexing, both triploid and diploid fish were grouped into all-male, all-female and mixed-sex groups, then reared in hapa at the density of 10 fish m^{-2} for 4 months and three replications for each group were provided. The highest body weight and body length gains and growth rate were obtained in all-male triploid fish, while the lowest of those parameters were obtained in all-female diploid fish. Highest survival rate was obtained in both all-male and mixed-sex triploids groups which no significantly different with the mixed-sex diploid group. Furthermore, triploid showed higher edible carcass percentage compared to diploid. Proximate analysis indicated that protein content of triploid was higher than of diploid, while lipid and ash contents were lower than of diploid. Triploid Nile tilapia have the best growth performances and quantity and quality of flesh than diploid.

Key words: Growth performance, triploid, diploid, monosex, mixed-sex, Nile tilapia

1. Introduction

Sterile fish is beneficial in aquaculture because in metabolism processes the fish will reduce or even prevent the use of energy for reproduction. As a result, whole anabolism energy will be transferred to somatic growth. Sterile fish was also potential for a better survival rate than diploid fish. Devlin et al. [1] stated that the increase in the growth of fish brings substantial benefits in shortening culture period, improving the efficiency of feed utilization, improving the efficiency of production and ensuring products availability. In addition, a culture of sterile fish is one of the best farming management in aquaculture practices, since it enables the use of the metabolism pathway to gain faster somatic tissue than production either sperm or eggs at the spawning season [2].

The high ability and uncontrolled of tilapia reproduction cause unexpected high density in the pond with varied size and slow growth, making it less commercially profitable in aquaculture. Sterilization is the best possible solution to solve the problems in the tilapia culture [3]. Lutz [4] mentioned that among future's aquaculture commodities, tilapia is a candidate fish species to produce functionally sterile seeds on a large scale. Induction of triploidy is one of the methods to produce sterile fish. The culture of triploid fish could provide benefits, such as increased growth, carcass production, survival rate and flesh quality [5,6,7].

Production of triploid tilapia has been developed for more than four decades and triploidy is an effective management tool in tilapia farming for the future [8]. Triploid tilapia has smaller size of testis or ovaries, lower gonad weight and higher body weight, protein utilization and protein efficiency ratio than diploid tilapia; thereby the farming is possibly beneficial [9]. In some cases, the growth performances of triploid tilapia were reported to be superior or equal to diploid tilapia relatively [10,11,12].

On the other hand, some tests indicated that male tilapia has faster growth than female tilapia [13,14,15]. The production level of male monosex tilapia farming was 10% higher when compared to mixed-sex population [16,17]. Associated with presence of sexual dimorphism in terms of growth, many efforts were made to produce all-male seed population for the purpose of monosex culture, which generally can be obtained by using four common ways, namely manual sexing [18] at 5-7 cm body size, hybridization [7,19], hormonal treatments [15,20,21,22,23,24,25,26,27] or chromosome set manipulations, such as androgenesis [18,28] to produce YY supermale parent stocks [29,30,31].

So far, the combined effects of triploidy and growth-related sexual dimorphism superiorities in tilapia are still unknown. A strain of fish species, include tilapia also possibly influence growth performance during the culture period. Therefore, in this study, we try to clarify the effect of those superiorities on growth, survival, flesh percentage and proximate composition of NIRWANA during the grow-out period.

2. Materials and methods

2.1. Experimental fish preparation

Fish that used in this study was Nile tilapia strain Wanayasa (NIRWANA) produced by families selection program of genetic improvement for farmed tilapia (GIFT) and genetically enhanced tilapia (GET) in Indonesia. Broodstocks were obtained from Tilapia and Common Carp Aquaculture Development Agency at Purwakarta, West Java, Indonesia. Artificially fertilized embryos (4 min after insemination) were subjected to heat shock treatment at 41^{o}C for 4 min to produce triploid fish. This treatment produced 91-100% triploid NIRWANA fish as identified using chromosome counting prepared according to Kligerman and Bloom [32] and Mukti et al. [33]. Embryos were incubated in glass funnel with a closed water recirculation system. Using similar procedure diploid fish were also produced.

Larvae of both triploid and diploid were separately reared in the 50-liter aquarium at a density of 1 fish l^{-1}, 10 aquaria were used for triploid and diploid fish, respectively. Fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Then, fish were transferred into 180-liter aquaria and reared at a density of 50 fish per aquarium, fed on a commercial diet (40% crude protein content) for 1 month. Sexing was conducted morphologically by observing anus, urethra, and genital openings, to separate male and female of both triploid and diploid fish. Twenty fish of different groups, namely all-male triploid, all-female triploid, mixed-sex triploid, all-male diploid, all-female diploid and mixed-sex diploid, respectively were prepared for performances evaluation.

2.2. Performances evaluation

Previously prepared all-male, all-female and mixed-sex of both triploid and diploid fish groups were separately transferred and reared in 2.0×1.0×0.7 m^3 floating net (mesh size 10 mm) placed in a 20×10×1.5 m^3 concrete pond at a density of 10 fish m^{-2}. Three floating nets for each group were used as replication. During the first month of rearing period, the fish were fed on a 1-mm-diameter commercial diet (40% crude protein content), and during the rest 3 months rearing period, the fish were fed on a 3-mm-diameter commercial diet (33% crude protein content) at satiation, three times a day.

Sexes of fish were checked at the monthly sampling time. Body weight, body length, survival rate and consumed feed data were collected every month, while dressing, fillet and proximate data of male and female, both triploid and diploid fish were analyzed at the end of the experiment. Dressing and fillet values were determined according to Buchtova et al. [34] and flesh proximate analysis was evaluated according to AOAC [35] based on ten samples of male and female, both triploid and diploid fish, respectively.

2.3. Statistical analysis

Data were analyzed statistically using analysis of variance (ANOVA) with SPSS ver.10 software. Significant ANOVA was followed by Duncan's multiple range test.

3. Results

3.1. Growth performance, survival rate, and feed conversion ratio

Growth performances of tested fish groups were shown in Table 1. The results showed that the growth of triploid fish was significantly higher ($P<0.05$) than diploid. Biomass gain (Δ B 3N-2N) of all-male, all-female, and mixed-sex triploids fish was 31.3, 11.4, and 23.4% higher than those of diploid counterparts, respectively. Similar pattern was found in body weight gain (Δ BW 3N-2N) and body length gain (Δ BL 3N-2N); the highest value (26.8 and 14.3%, respectively) were shown in all-male triploid, followed by mixed-sex triploid and the lowest value (9.6 and 6.2%, respectively) were shown in all-female triploid. Furthermore, all-female diploid fish significantly showed the lowest growth performance compared to other groups.

Absolute growth rate (AGR) and survival rate of both all-male and mixed-sex triploid fish were higher than other groups. All-male triploid has highest AGR than other groups, followed by mixed-sex triploid, then by all-male and all-female diploids, while mixed-sex triploid has a best feed conversion ratio, followed by all-male triploid and diploid. The survival rates of all-male and mixed-sex triploids and mixed-sex diploid were higher compared to other groups as shown in Table 1.

Figure 1 shows monthly body weight and body length recorded during 4 months grow-out period. In general, triploid grew faster than diploid and all-male triploid showed the highest growth rate, while all-female diploid showed the lowest growth rate.

In this study, we noted that in both triploid and diploid fish, male grew faster than female during the experiment; in triploid and diploid groups, biomass gain of the male were 55.5 and 31.9% higher than female, respectively. Based on average body weight, before maturation period, triploid and diploid males showed 16.6 and 10.7 g higher than females, respectively, while during maturation period, triploid and diploid males showed 103.3 and 50.5 g higher than females, respectively. These results showed that the role of sexual dimorphism on growth in Nile tilapia fish has a similar pattern with the role of ploidy level, by which their effects were highly significant during the maturation period.

At 90-day-old fish, all-female and mixed-sex triploids showed the same growth rate. At 120- to 180-day-old fish, mixed-sex triploid showed higher specific growth rate (SGR); while at 120-day-old fish, all-female triploid showed same SGR as all-male diploid. At 150-days-old fish, all-female triploid, all-male and mixed-sex diploids showed same SGR. At 180-days-old fish, all-female triploid showed the same SGR as mixed-sex diploid (Figure 2).

3.2. Flesh percentage and proximate composition

Fillet percentages of male and female triploids were higher than diploids, highest and lowest dressing percentages were found in female triploid and female diploid, respectively ($P<0.05$). Dressing and fillet percentages of female triploid were 8.6 and 10.5% higher than female diploid, respectively, while male triploid was 2.1 and 5.9% higher than male diploid, respectively (Table 2).

Flesh proximate analysis of triploid and diploid fish is shown in Table 3. The protein content of female triploid was same as male triploid but higher than diploid fish ($P<0.05$). On the other hand, lipid and ash contents of male and female triploids were lower than diploids. There are no significant differences in carbohydrate content between triploid and diploid fish.

4. Discussion

This study revealed that there is an important role of ploidy level and sexual dimorphism on the Nile tilapia fish growth performance. Highest growth of male triploid and lowest growth of female diploid indicated that both ploidy level and sexual dimorphism significantly contribute their effects on Nile tilapia fish growth (Table 1 and Figures 1 and 2).

Tave [36] reported that triploidization lead to increase sterility and growth. A cell size of triploid is larger than diploid and energy for gamete production is reduced or inhibited. In most cases, triploid showed heavier body size and faster growth than diploid during 110 days grow-out period in common carp (*Cyprinus carpio*) [37], during 8 weeks in African mud catfish (*Clarias gariepinus*) [38], during 175 days in Chinese catfish (*C. fuscus*) [39] and during 12 weeks in Atlantic salmon (*Salmo salar*) [40]. In addition, performances of triploid fish were not only species and age-dependent, but also depend on experimental conditions and interactions between the environment and genetics [7]. Individually body size of triploid was larger due to their larger cell size than diploid [41]. However, Aliah et al. [42] reported that the cell size was not correlated with the organ size on sticklebacks (*Gasterosteus aculeatus*). Furthermore, in 2- to 3-month-old sunshine bass (*Morone* spp.), diploid grew faster compared to triploid [43].

Increasing of triploid tilapia growth is possibly due to the sterility influence, which it can divert nutrient energy for somatic growth rather than gonadal development and sexual activity [14]. Most studies concluded that significantly different growth rate between triploid and diploid fish occurs at maturation period, such as in turbot (*Scophthalmus maximus*) [44] and European sea bass (*Dicentrarchus labrax*) [45]. In this study, we found that the growth difference (30.0%) between triploid and diploid fish has already occurred before maturation period (≤ 90-days-old) and during maturation period (90- to 180-day-old), the growth of triploid showed more significantly different than diploid (39.3%). A similar phenomenon has been reported on fancy carp (*C. carpio*) [46].

The role of sexual dimorphism on growth in tilapia has been revealed since more than three decades. Male tilapia grew faster compared to female, so the all-male monosex culture in this species is worldwide applied. Similar cases were found by most researchers in catfish (*C. gariepinus*) [47] and crucian carp (*Carassius auratus*) [48].

Comparative of growth performance among six groups showed that all-male triploid and all-female diploid fish grew fastest and lowest than other groups, respectively during the experiment. At 120- to 150-day-old fish, the interaction effect of triploidy and sexual dimorphism on growth was not significantly differenced among all-female triploid, all-male diploid and mixed-sex diploid. At 180-day-old, among these groups, all-male diploid grew faster than the others and interaction effect of triploidy and sexual dimorphism on growth was not significantly different among all-female triploid and mixed-sex diploid (Figure 2). This phenomenon seems to be species-specific, as found in rainbow trout (*Oncorhynchus mykiss*) by Tabata et al. [49], Mozambique tilapia (*O. mossambicus*) by Varadaraj and Pandian [50] and European sea bass by Felip et al. [51] who reported that female triploid grew faster than either male triploid, male and female diploid or mixed-sex diploid.

Lowest growth of all-female diploid fish than other groups looked as if female diploid undergo faster reproductive development and sexual maturity, so the utilization of available energy might be more allocate for gonadal development or gametogenesis than somatic growth. In this study, we recorded that at 120-day-old fish, a majority of female diploid fish began to spawn and incubate either fertilized or unfertilized eggs in the mouth. This generally allowed female fish never eat during eggs incubation for 15 days until larvae have been able to swim freely as reported by Byamungu et al. [52]. In other words, the role of ploidy level on growth during maturation period was significantly higher than before the maturation period. These results also revealed that a higher body weight gain of male and female triploid than diploid during maturation period seem due to the sterility of triploid fish and reproductive activity of diploid fish.

In this study, triploid fish indicated higher flesh percentages than diploid and female triploid showed higher flesh percentages. Similar results were reported in gilthead sea bream (*Sparus aurata*) [53] and rainbow trout [54]. However, in common carp [55] up to size 400 g, dressing weight of triploid was not significantly different than diploid. Our result indicated that higher flesh percentages of female triploid than male triploid due to the female more sterile than male, while the higher flesh percentages of triploid than diploid seem correlated with normal in diploid and reducing in triploid of gonadal developments.

Triploid Nile tilapia fish tends to be higher protein content and lower lipid and ash contents than diploid. By sex, male and female fish both in triploid and diploid showed the same content of protein, lipid, and carbohydrate, while the ash content was significantly different. This result showed that triploidy in Nile tilapia affects flesh qualities, especially lipid and ash contents. Further study is needed to achieve more valuable information.

Interaction effect of triploidy and sexual dimorphism related growth strongly gives a positive contribution on production performance, especially during the maturation period. Based on examination of varies aspects related to production, the result revealed that all-male triploid Nile tilapia culture is prospective to be developed. Hence, in the future, an applicable method for mass all-male triploid seed production should be considered. One of the possible strategic efforts is how to produce supermale tetraploid as parent stock by combining the technology of chromosome set and hormonal manipulations.

Acknowledgments

This study was partially supported by the Ministry of Research, Technology and Higher Education, the Republic of Indonesia through BPP-DN scholarship program and Post-Doctoral Research Grant. We wish to thank the deceased Prof. Komar Sumantadinata who has provided guidance and support during the study period, and the Head and Staffs of Tilapia and Common Carp Aquaculture Development Agency at Purwakarta, West Java, Indonesia on aid provision NIRWANA Broodstocks.

References

1. Devlin R, Biagi CA, Yesaki TY. Growth, viability and genetic characteristics of GH transgenic Coho salmon strains. Aquaculture 2004; 236: 607–632.
2. Galli L. Genetic modification in aquaculture - a review of potential benefits and risks. Bureau of Rural Sciences, Canberra, Australia. 2002.
3. Pradeep PJ, Srijaya TC, Jose D, Papini A, Hassan A, Chatterji AK. Identification of diploid and triploid red tilapia by using erythrocyte indices. Caryologia 2011; 64: 485–492.
4. Lutz CG. Practical genetics for aquaculture. Fishing News Books, Blackwell Science, Oxford. 2001.
5. Felip A, Zanuy S, Carrillo M, Piferrer F. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 2001a; 111: 175–195.

6. Melamed P, Gong Z, Fletcher G, Hew CL. The potential impact of modern biotechnology on fish aquaculture. Aquaculture 2002; 204: 255–269.

7. Dunham RA. Aquaculture and fisheries biotechnology: genetic approaches. CABI Publishing, Cambridge. 2004.

8. Pradeep PJ, Srijaya TC, Bahuleyan A, Papini A. Can sterility through triploidy induction make an impact on Tilapia industry? International Journal of Aquatic Science 2012a; 3: 89–96.

9. Pechsiri J, Yakupitiyage A. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 2005; 36: 45–51.

10. Mol K, Byamungu N, Cuisset B, Yaron Z, Ofir M, Melard Ch, Castelli M, Kuhn ER. Hormonal profile of growing male and female diploids and triploids of the blue tilapia (*Oreochromis aureus*) reared in intensive culture. Fish Physiology and Biochemical 1994; 13 (3): 209–218.

11. Hussain MG, Rao GPS, Humayun NM, Randall CF, Penman DJ, Kime D, Bromage NR, Myers JM, McAndrew BJ. Comparative performance of growth, biochemical composition and endocrine profiles in diploid and triploid tilapia (*Oreochromis niloticus* L.). Aquaculture 1995; 138: 87–97.

12. Puckhaber B, Horstgen-Schwark G. Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). In: Pullin RSV, Lazard M, Legendre JB, Kothlas A, Pauly D (eds): The Third International Symposium on Tilapia in Aquaculture. Manila: ICLARM Conference Proceedings. 1996; pp. 377–382.

13. Bhatta S, Iwai T, Miura T, Huguchi M, Maugars G, Chiemi M. Differences between male and female growth and sexual maturation in tilapia (*Oreochromis*

mossambicus). Kathmandu University Journal of Science, Engineering and Technology 2012; 8: 57–65.

14. Pradeep PJ, Srijaya TC, Papini A, Chatterji AK. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture 2012b; 344–349: 181–187.
15. Fuentes-Silva C, Soto-Zarazua GM, Torres-Pacheco I, Flores-Rangel A. Male tilapia production techniques: a mini-review. African Journal of Biotechnology 2013; 12: 5496–5502.
16. Nguyen CD, David CL. The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in Northern Vietnam. Aquaculture 2000; 184: 221–231.
17. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. Journal of Endocrinology 2001; 170: 27–38.
18. Cnaani A, Levavi-Sivan B. Sexual development in fish: practical applications for aquaculture. Sex Development 2009; 3: 164–175.
19. Bartley D, Rana K, Immink A. The use of interspecific hybrids in aquaculture and fisheries. Review Fish Biology and Fisheries 2001; 10: 325–337.
20. Popma TJ, Green BW. Sex reversal of tilapia in earthen ponds. Aquaculture Production Manual, Research and Development Series No. 35, International Center for Aquaculture, Auburn University, Alabama, USA. 1991.
21. Pandian TJ, Sheela SG. Hormonal induction of sex reversal in fish. Aquaculture 1995; 138: 1–22.

22. Mukti AT. Optimation of 17α-methyltestosterone synthetic hormone dose and immersion duration in larvae on the success of Nile tilapia (*Oreochromis* sp.) sex reversal. Faculty of Fisheries, Brawijaya University, Malang, Indonesia. 1998.

23. Romerio MP, Fencrich-Verani CSN, Santo De-Copmus BE, Pasilva AS. Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasil Zoology 2000; 29: 654–659.

24. Mukti AT, Priyambodo B, Rustidja, Widodo MS. Optimization of both 17α-methyltestosterone synthetic hormone dosage and dipping duration of Nile tilapia (*Oreochromis* sp.) larvae on sex reversal efficacy. BIOSAIN Journal of Life Science 2002; 2 (1): 1–8.

25. Asaad HM, Traifalgar RFM, Serrano Jr. AE, Peralta JP, Pedroso FL. Dietary administration of dehydroepiandrosterone hormone influences the sex differentiation of hybrid red Tilapia (*O. niloticus* × *O. mossambicus*) larvae. Journal of Fisheries and Aquatic Science 2012; 7: 447–453.

26. Beaven U, Muposhi V. Aspects of a monosex population of (*Oreochromis niloticus*) fingerling produced using 17-α methyl testosterone hormone. Aquaculture Research and Development 2012; 3: 3.

27. Dagne A, Degefu F, Lakew A. Comparative growth performance of monosex and mixed-sex Nile tilapia (*Oreochromis niloticus* L.) in pond culture system at Sebeta, Ethiopian. International Journal of Aquaculture 2013; 7: 30–34.

28. Ezaz MT, Myers JM, Powell SF, McAndrew BJ, Penman DJ. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 2004; 232: 205–214.

29. Muller-Belecke A, Horstgen-Schwark G. A YY-male (*Oreochromis niloticus*) strain developed from an exceptional mitotic gynogenetic male and growth performance testing of genetically all-male progenies. Aquaculture Research 2007; 38: 773–775.

30. Aliah RS, Sumantadinata K, Maskur, Naim S. GESIT tilapia: Indonesia's genetic supermales. Global Aquaculture Advocate. May/June. 2010; 36–37.

31. Turra EM, Oliveira DAA, Teixeira EA, Luz RK, Prado SA, Melo DC, Faria PMC, Sousa AB. Reproduction control in Nile tilapia (*Oreochromis niloticus*) by sexual and chromosome set manipulation. Revista Brasil de Reproduction Animal, Belo Horizonte. 2010; 34: 21–28.

32. Kligerman AD, Bloom SE. Rapid chromosome preparation from solid tissues of fish. Journal of Fisheries Research Board Canada. 1977; 34: 266–269.

33. Mukti AT, Carman O, Alimuddin, Zairin Jr. M. A rapid chromosome preparation technique without metaphase arrest for ploidy determination in Nile tilapia (*Oreochromis niloticus*). Caryologia 2016. doi: 10.1080/00087114.2016.1152112.

34. Buchtova H, Svobodova Z, Kocour M, Velisek J. Evaluation of the dressing percentage of 3-year-old experimental scaly crossbreds of the common carp *Cyprinus carpio* (Linnaeus, 1758) in relation to sex. Acta Veterinary Brno 2006; 75: 123–132.

35. AOAC [Association of Official Analytical Chemists]. Official methods of analysis. (18th eds), Association of Official Analytical Chemists Inc., Washington. 2005.

36. Tave D. Genetics for fish hatchery managers. Avi Publishing, Connecticut. 1993.

37. Mukti AT, Rustidja, Sumitro SB, Djati MS. Polyploidization of common carp (*Cyprinus carpio* L.). BIOSAIN Journal of Life Science 2001; 1: 111–123.

38. Lawson EO, Ishola HA. Effects of cold shock treatment on the survival of fertilized eggs and growth performance of the larvae of African mud catfish *Clarias gariepinus* (Burchell, 1822). Research Journal of Fisheries Hydrobiology 2010; 5: 85–91.

39. Qin JG, Fast AW, Ako H. Grow-out performance of diploid and triploid Chinese catfish (*Clarias fuscus*). Aquaculture 1998; 166: 247–258.

40. Burke HA, Sacobie CFD, Lall SP, Benfey TJ. The effect of triploidy on juvenile Atlantic salmon (*Salmo salar*) response to varying levels of dietary phosphorus. Aquaculture 2010; 306: 295–301.

41. Piferrer F, Beaumont A, Falguière J-C, Flajšhans M, Haffray P, Colombo L. Polyploid fish and shellfish: Production, biology, and applications to aquaculture for performance improvement and genetic containment. Aquaculture 2009; 293: 125–156.

42. Aliah RS, Yamaoka K, Inada Y, Taniguchi N. Effects of triploidy on tissue structure of some organs in ayu. Bulletin Japan Society Science Fisheries 1990; 56: 569–575.

43. Kerby JH, Eversona JM, Harrell RM, Geiger JG, Starling CC, Revels H. Performance comparisons between diploid and triploid sunshine bass in freshwater ponds. Aquaculture 2002; 211: 91–108.

44. Cal RM, Vidal S, Gomez C, Ivarez-Blazquez BA, Martinez P, Piferrer F. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). Aquaculture 2006; 251: 99–108.

45. Felip A, Piferrer F, Zanuy S, Carrillo M. Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons. Journal of Fish Biology 2001b; 58: 76–88.

46. Taniguchi N, Kijima A, Tamura T, Takegami K, Yamasaki I. Color, growth and maturation in ploidy-manipulated fancy carp. Aquaculture 1986; 57: 321–328.

47. Achegbulu CE, Okonji VA, Obi A. Growth and economic performance of diploid and triploid African catfish (*Clarias gariepinus*) in outdoor concrete tanks. International Journal of Genetics 2013; 3: 1–6.

48. Chen S, Wang J, Liu SJ, Qin QB, Xiao J, Duan W, Luo KK, Liu JH, Liu Y. Biological characteristics of an improved triploid crucian carp. Science China Series C: Life Science 2009; 52: 733–738.

49. Tabata YA, Rigolino MG, Tsukamoto RY. Production of all-female triploid rainbow trout (*Oncorhynchus mykiss*) [Pisces, Salmonidae]. III. Growth up to first sexual maturation. Boletim do Instituto de Pesca Sao Paulo 1999; 25: 67–76.

50. Varadaraj K, Pandian TJ. Production of all-female sterile-triploid (*Oreochromis mossambicus*). Aquaculture 1990; 84: 117–123.

51. Felip A, Carrillo M, Zanuy S. Older triploid fish retain impaired reproductive endocrinology in the European sea bass (*Dicentrarchus labrax*). Journal of Fish Biology 2009; 75: 2657–2669.

52. Byamungu N, Darras VM, Kuhn ER. The growth of heat-shock induced triploids of blue tilapia (*Oreochromis aureus*) reared in tanks and in ponds in Eastern Congo: feeding regimes and compensatory growth response of triploid females. Aquaculture 2001; 198: 109–122.

53. Haffray P, Bruant J-S, Facqueur J-M, Fostier A. Gonad development, growth, survival and quality traits in triploids of the protandrous hermaphrodite gilthead sea bream (*Sparus aurata* L.). Aquaculture 2005; 247: 107–117.

54. Werner C, Poontawee K, Mueller-Belecke A, Horstgen-Schwark G, Wicke M. Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. Archiv Tierzucht 2008; 51: 71–83.

55. Basavaraju Y, Mair GC, Kumar HMM, Kumar SP, Keshavappa GY, Penman DJ. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp (*Cyprinus carpio*) in Karnataka, India. Aquaculture 2002; 204: 407–418.

Table 1. The growth, survival rate and feed conversion ratio performances of all-male, all-female and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period (n = 20).

Parameter	Fish group					
	Triploid			Diploid		
	All-male	All-female	Mixed-sex	All-male	All-female	Mixed-sex
Initial biomass (g)	278.6±5.2	190.0±8.3	236.2±6.0	205.0±8.9	136.0±8.8	183.4±5.8
Final biomass (g)	8 056.7±405.5	5 193.3±445.6	7 013.3±551.4	6 130.0±366.6	4 626.7±277.6	5 676.7±465.0
Δ Biomass (g)	7 778.1±404.3^{a}	5 003.0 ±437.9^{e}	6 777.1±548.9^{b}	5 925.0±363.5^{c}	4 490.7±284.9^{f}	5 493.2±462.9^{d}
Δ B 3N-2N (%)	31.3	11.4	23.4	-	-	-
Initial BW (g)	13.9±0.3	9.5±0.4	11.8±0.3	10.3±0.4	6.8±0.4	9.2±0.3
Final BW (g)	402.8±20.3	278.5±23.2	350.7±27.6	317.0±13.5	252.3±10.2	288.3±15.5
Δ BW (g)	388.9±20.2^{a}	269.0±22.8^{d}	338.9±27.4^{b}	306.7±13.6^{c}	245.5±10.7^{e}	279.2±15.3^{d}
Δ BW 3N-2 N (%)	26.8	9.6	21.4	-	-	-
Initial BL (mm)	99.2±0.0	93.3±0.0	92.5±0.0	96.3±0.0	92.8±0.0	91.2±0.0
Final BL (mm)	274.5±2.1	241.3±6.7	266.5±5.6	250.0±2.4	232.2±1.9	243.4±4.6
Δ BL (mm)	175.7±2.1^{a}	147.9±6.7^{d}	174.0±5.6^{b}	153.7±2.4^{c}	139.3±1.9^{e}	152.2±4.6^{c}
Δ BL 3N-2N (%)	14.3	6.2	14.3	-	-	-
AGR (g day^{-1})	3.2±0.2^{a}	2.2±0.2^{d}	2.8±0.2^{b}	2.6±0.1^{c}	2.1±0.1^{e}	2.3±0.1^{d}
Condition factor	1.9±0.1^{b}	2.0±0.0^{bc}	1.9±0.0^{a}	2.0±0.1^{c}	2.0±0.0^{c}	2.0±0.0^{bc}
Feed consumption (g)	470.3±13.8^{c}	367.8±2.0^{b}	378.7±21.5^{b}	380.3±7.0^{b}	341.4±7.4^{a}	378.5±27.3^{b}
Feed conversion ratio	1.2±0.1^{b}	1.4±0.1^{c}	1.1±0.0^{a}	1.2±0.1^{b}	1.4±0.0^{c}	1.4±0.0^{c}
Survival rate (%)	100.0±0.0^{a}	93.3±5.8^{c}	100.0±0.0^{a}	96.7±2.9^{b}	91.7±2.9^{c}	98.3±2.9^{ab}

Note: Δ: gain, Δ B 3N-2N: relative percentage of triploid:diploid biomass gain, BW: body weight, Δ BW 3N-2N: relative percentage of triploid:diploid body weight gain, BL: body length, Δ BL 3N-2N: relative percentage of triploid:diploid body length gain, AGR: absolute growth rate and Feed consumption: amount of given feed. Different superscript in the same row indicates significant differences ($P<0.05$)

Table 2. Flesh percentages of male and female triploid and diploid Nile tilapia fish (n = 10).

Fish group		Body weight (g)	Dressing		Fillet	
			Weight (g)	(%)	Weight (g)	(%)
Triploid	♂	414.1±39.2[a]	238.3±19.9[a]	57.6±1.8[b]	170.9±16.0[a]	41.3±1.4[a]
	♀	260.8±24.0[c]	154.0±13.5[c]	59.1±1.6[a]	109.4±10.8[c]	42.0±1.2[a]
Diploid	♂	332.0±29.7[b]	187.2±18.4[b]	56.4±1.6[b]	129.4±12.4[b]	39.0±1.6[b]
	♀	259.4±14.1[c]	141.0±7.8[c]	54.4±1.3[c]	98.5±6.0[d]	38.0±1.4[b]

Note: Different superscript in the same column indicates significant differences ($P<0.05$)

Table 3. Flesh proximate analysis of male and female triploid and diploid Nile tilapia fish (% dry weight) (n = 10).

Fish group		Protein	Lipid	Ash	Carbohydrate
Triploid	♂	85.6±0.3[ab]	5.1±0.2[b]	6.2±0.2[c]	3.2±0.7[a]
	♀	87.0±1.1[a]	5.0±0.4[b]	5.9±0.0[d]	2.2±1.5[a]
Diploid	♂	84.2±1.3[b]	5.9±0.3[a]	7.1±0.0[a]	2.8±1.7[a]
	♀	84.3±1.8[b]	5.5±0.0[a]	6.4±0.3[b]	3.8±1.5[a]

Note: Different superscript in the same column indicates significant differences (P<0.05)

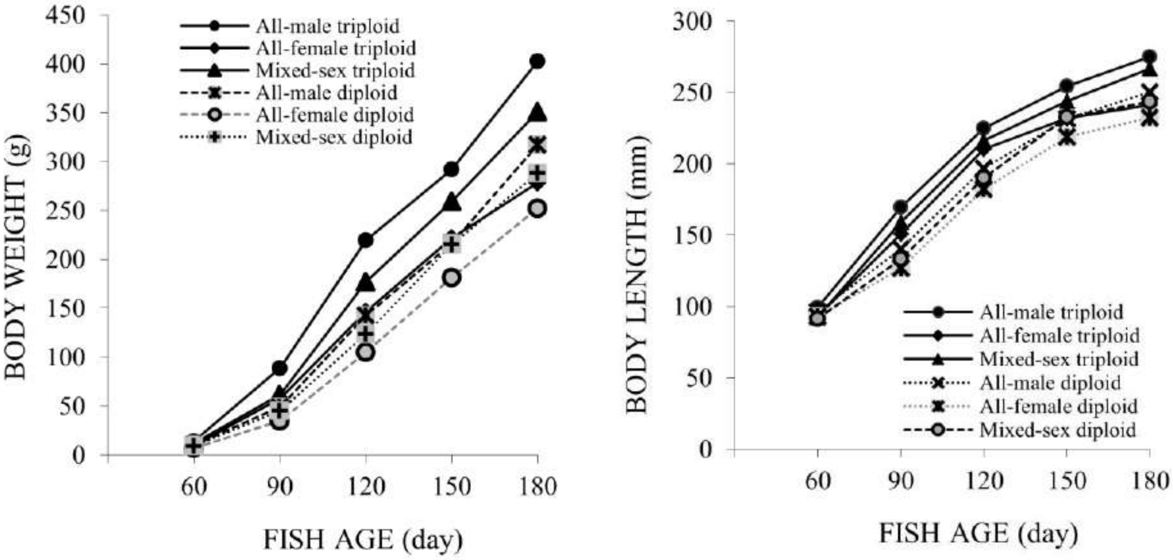

Figure 1. Body weight and body length of all-male, all-female and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period

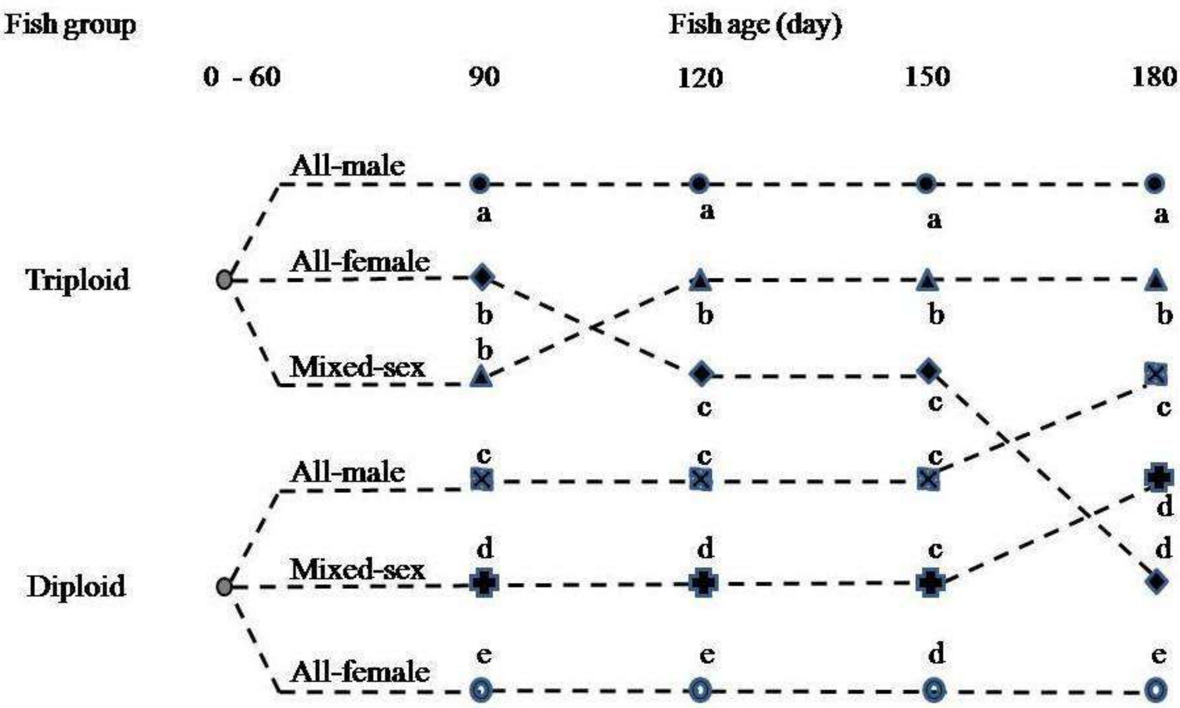

Figure 2. Schematic sequential specific growth rates of triploid and diploid Nile tilapia fish during 4 months grow-out period. Different letter at the same fish age indicates significant differences (P<0.05)

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Selasa, 9 Juli 2019 16.14 GMT+7

Dear AKHMAD MUKTI

Your manuscript has been evaluated and has been found to have too many language errors as well as other deficiencies listed below regarding the submission rules. You are requested to correct these deficiencies and language errors and re-submit your manuscript within 30 days so that its processing may begin immediately.

Deficiencies:

1. English is not good enough, and must be revised.
2. The order of author names on the copyright release form must be the same as the order on the manuscript and the order entered into the on-line system during submission. (First Name, followed by Last Name)
3. Author(s) name(s) not given in full in the system/on the manuscript/on the Copyright Release Form (do not abbreviate)
4. Copyright Release Form completed and signed by the corresponding author has not been uploaded.
5. Other reasons (Look at technical comments)
6. A space must be inserted between numbers and symbols, such as x, =, <, >, +, -.
7. Manuscript not prepared according to the instructions

Technical Comments: Until your manuscript complies with all of the journal's rules it will not be processed further.
--- You must write authors ORCid's to the cover page. (for all authors). You can visit https://orcid.org/ to get your unique ORCID IDs.
--Do not abbreviate names of journals in the end reference list. Names of journals must be written out in full
-In the end reference list, et al. must be used after the first 5 authors of a publication. Give only the names of the first 5 authors, and then et al.
-In the references section use a hyphen between page numbers and not an en dash, e.g., 114-119 and not 114?119.
Please insert a space between the numbers and degree symbols in your manuscript.

We thank you for your interest in our journal.

Yours sincerely,

YÜCEL UYAR
Journal Administrator

Article Title: Growth performance, survival, flesh percentage, and proximate composition of mono- and mixed-sex-cultured triploid and diploid Nile tilapia (Oreochromis niloticus)
Article Code Number: VET-1905-79
Web address: http://online.journals.tubitak.gov.tr

NOTE: To resubmit your manuscript, log onto the online system as corresponding author and find your manuscript in the 'Manuscripts Sent Back to Author Because of Deficiencies' list. Then you can resubmit your manuscript. Please do not use 'Submit New Manuscript' link for resubmission.

English Editors' Note:

The paper you have submitted for publication has been evaluated by the English editors, and the English has been determined to be below the acceptable level. We therefore regret to inform you that it is not possible for your paper to be considered for publication in its present form.

Because of the nature of the errors, which involve serious problems with sentence structure and grammar, we do not recommend that you attempt to make corrections yourself, but that you have someone else, preferably a native speaker, make corrections.

Our department receives a yearly average of 3,500 papers for consideration. For this reason, it is impossible for us to mark specific mistakes. The areas in your paper that need to be corrected should be clear to a native speaker, or to someone with advanced knowledge of English.

Yours sincerely,

The Department of Scientific Journals

Growth performance, survival rate, flesh, and proximate composition of mono- and mixed-sex triploid and diploid Nile tilapia (*Oreochromis niloticus*)

Abstract: This study aimed to compare the growth performance, survival rate, flesh, and proximate composition of both mono- and mixed-sex triploid and diploid Nile tilapia. The triploid population was obtained through heat shock at 41 ºC for 4 minutes, 4 minutes after fertilization. Before sexing, fish were reared in aquariums at a density of 50 fish/aquarium for 2 months. After sexing, both triploid and diploid fish were grouped into all-male, all-female, and mixed-sex groups and reared in hapa at a density of 10 fish/m^2 for 4 months. Each group was replicated three times. The highest body weight, body length, and growth rate were observed in all-male triploid fish, while the lowest values of those parameters were obtained in all-female diploid fish. The highest survival rate was achieved in both all-male and mixed-sex triploids groups and did not significantly differ to the mixed-sex diploid group. Furthermore, the triploid fish had higher edible carcass percentage compared to diploid. The proximate analysis indicated that the protein content of triploid was higher than that of diploid, while the lipid and ash contents were lower than those of diploid. Triploid Nile tilapia had the best growth performances, including quantity and quality of flesh compared to diploid.

Key words: Growth performance, triploid production, monosex, mixed-sex, Nile tilapia

1. Introduction

Sterile fish is beneficial in aquaculture because, in the sterile metabolism processes, the fish will reduce or even prevent the use of energy for reproduction. As a result, most of the anabolic energy will be transferred to somatic growth. Sterile fish also has the potential for a better survival rate compared to diploid fish. Devlin et al. [1] stated that the increase in the growth of fish brings substantial benefits in shortening culture period, improving the efficiency of feed utilization and the efficiency of production, and ensuring product availability. Also, culturing sterile fish is one of the best farming management in aquaculture practices, as it enables the use of the metabolism pathway to reach fast somatic tissue instead of producing either sperm or eggs in the spawning season [2].

The high ability (uncontrolled) of tilapia reproduction cause the unexpected density in the pond with varied size and slow growth, making it less commercially profitable in aquaculture. The sterilization is the best possible solution to solve the problems in the tilapia culture [3]. Lutz [4] mentioned that among future's aquaculture commodities, tilapia is a candidate fish to produce functionally sterile seeds on a large scale. The induction of triploidy is one of the methods of producing sterile fish. The culture of triploid fish could provide benefits, such as increased growth, carcass production, survival rate, and flesh quality [5,6,7].

The production of triploid tilapia has been developed for more than four decades, and triploidy is an effective management tool in tilapia farming in the future [8]. Triploid tilapia has small testis or ovaries, low gonad weight and high body weight, protein utilization, and protein efficiency ratio compared to diploid tilapia. Thus,

farming is possibly beneficial [9]. In some cases, the growth performances of triploid tilapia were reported to be superior or equal to those of diploid tilapia [10,11,12].

On the other hand, some tests indicated that male tilapia has faster growth compared to female tilapia [13,14,15]. The production level of monosex male tilapia farming was 10% higher compared to the mixed-sex population [16,17]. Associated with presence of sexual dimorphism in terms of growth, many efforts were made to produce all-male seed population for the purpose of monosex culture, which generally can be obtained through four common methods, namely manual sexing [18] at body size of 5-7 cm, hybridization [7,19], hormonal treatments [15,20,21,22,23,24,25,26,27] or chromosome set manipulations, such as androgenesis [18,28] to produce YY supermale parent stocks [29,30,31].

So far, the combined effects of triploidy and growth-related sexual dimorphism superiorities in tilapia are still unknown. A strain of fish, including tilapia, also possibly influence growth performance during the culture period. Therefore, the present study tries to clarify the effect of those superiorities on growth, survival rate, flesh percentage, and proximate composition of Nile tilapia during the grow-out period.

2. Materials and methods

2.1. Experimental fish preparation

Fish used in this study was Nile tilapia strain Wanayasa (NIRWANA) produced through family selection program of genetic improvement for farmed tilapia (GIFT) and genetically enhanced tilapia (GET) in Indonesia. The broodstocks were obtained from the Tilapia and Common Carp Aquaculture Development Agency in Purwakarta, West Java, Indonesia. Artificially fertilized embryos (4 minutes after insemination) were subjected to heat shock treatment at 41 ºC for 4 minutes to produce triploid fish. This treatment produced 91 - 100% triploid Nile tilapia fish as identified using the chromosome counting method prepared according to Kligerman and Bloom [32] and Mukti et al. [33]. Embryos were incubated in glass funnel in a closed water recirculation system, and diploid fish were produced using a similar procedure.

Larvae of both triploid and diploid were separately reared in 50 - L aquariums at a density of 1 fish L^{-1}. A total of 10 aquariums were used for triploid and diploid fish, respectively. Fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Next, fish were transferred into 180 - L aquariums, reared at a density of 50 fish per aquarium and fed on a commercial diet (40% crude protein content) for 1 month. Sexing was conducted morphologically by observing the anus, urethra, and genital openings, to separate male and female of both triploid and diploid fish. Twenty fish from different groups, namely all-male triploid, all-female triploid, mixed-sex triploid, all-male diploid, all-female diploid and mixed-sex diploid, were respectively prepared for performance evaluation.

2.2. Performances evaluation

Previously prepared all-male, all-female, and mixed-sex of both triploid and diploid were separately transferred and reared in $2.0 \times 1.0 \times 0.7$ m^3 floating net (mesh size of 10 mm) placed in a $20 \times 10 \times 1.5$ m^3 concrete pond at a density of 10 fish m^{-2}. Three floating nets were used as replication for each group. During the first month of the rearing period, the fish were fed on a 1-mm-diameter commercial diet (40% crude protein content), while the fish were fed on a 3-mm-diameter commercial diet (33% crude protein content) at satiation during the last 3 months, three times a day.

The gender of the fish was checked at the monthly sampling time. Body weight, body length, survival rate and consumed feed data were collected every month, while dressing, fillet and proximate data of male and female both triploid and diploid fish were analyzed at the end of the experiment. The dressing and fillet data were determined according to Buchtova et al. [34], and flesh proximate analysis was evaluated according to AOAC [35] based on ten samples from male and female both triploid and diploid, respectively.

2.3. Statistical analysis

Data were statistically analyzed using the analysis of variance (ANOVA) with SPSS ver.10 software. Duncan's multiple range test followed the ANOVA test.

3. Results

3.1. Growth performance, survival rate, and feed conversion ratio

The growth performances of the tested fish groups are shown in Table 1. The results showed that the growth of triploid fish was significantly higher ($P < 0.05$) compared to that of diploid. The biomass gains (Δ B 3N - 2N) of all-male, all-female, and mixed-sex triploids fish were 31.3, 11.4, and 23.4% higher than those of diploid, respectively. A similar pattern was found in body weight gain (Δ BW 3N - 2N) and body length gain (Δ BL 3N - 2N). The highest values (26.8 and 14.3%, respectively) were observed in all-male triploid, followed by mixed-sex triploid, while the lowest values (9.6 and 6.2%, respectively) were seen in all-female triploid. Furthermore, all-female diploid fish significantly showed the most inferior growth performance compared to other groups.

The Absolute growth rate (AGR) and the survival rate of both all-male and mixed-sex triploid fish were higher than those of the other groups. All-male triploid had the highest AGR than other groups, followed by mixed-sex triploid, then all-male and all-female diploids. Meanwhile, the mixed-sex triploid had the best feed conversion ratio, followed by all-male triploid and diploid. The survival rates of all-male and mixed-sex triploids and mixed-sex diploid were higher compared to other groups, as shown in Table 1.

Figure 1 shows the monthly body weight and body length recorded during the 4 months grow-out period. In general, triploid grew faster than diploid, and all-male triploid showed the highest growth rate, while all-female diploid showed the lowest growth rate.

In this study, it was observed that in both triploid and diploid fish, male grew faster than female during the experiment. In triploid and diploid groups, the biomass gains of the male were 55.5 and 31.9% higher those of female, respectively. Based on the average body weight before the maturation period, triploid and diploid males had 16.6 and 10.7 g higher than females, respectively. Meanwhile, during the maturation period, triploid and diploid males had 103.3 and 50.5 g bigger than females, respectively. These results showed that the role of the sexual dimorphism on growth in Nile tilapia had a similar pattern with the role of the ploidy level, the effects of which were highly significant during the maturation period.

At 90 days old, all-female and mixed-sex triploids showed the same growth rate. At 120 to 180 days old, the mixed-sex triploid had higher specific growth rate (SGR), while at 120 days old all-female triploid had same SGR as all-male diploid. At 150 days old, all-female triploid, all-male and mixed-sex diploids had same SGR. At 180 days old, all-female triploid had the same SGR as the mixed-sex diploid (Figure 2).

3.2. Flesh percentage and proximate composition

The fillet percentages of male and female triploids were higher than those of diploids. The highest and lowest dressing percentages were found in female triploid and female diploid, respectively ($P < 0.05$). The dressing and fillet percentages of female triploid were 8.6 and 10.5%, respectively, which were higher than those of female diploid. Meanwhile, the dressing and fillet percentages of male triploids were 2.1 and 5.9% higher than those of the diploids, respectively (Table 2).

Flesh proximate analysis of triploid and diploid fish is shown in Table 3. The protein content of female triploid was similar to that of male triploid but higher than that of diploid fish ($P < 0.05$). On the other hand, lipid and ash contents of male and female triploids were lower than diploids. There were no significant differences in carbohydrate content between triploid and diploid fish.

4. Discussion

This study revealed that ploidy level and sexual dimorphism play essential roles in Nile tilapia growth performance. The high growth of male triploid and low growth of female diploid indicated that both ploidy level and sexual dimorphism significantly affected Nile tilapia growth (Table 1 and Figures 1 and 2).

Tave [36] reported that triploidization lead to increase in sterility and growth. A cell size of triploid is larger than diploid, and energy for gamete production is reduced or inhibited. In most cases, triploid showed heavier body size and faster growth than diploid during 110 days grow-out period in common carp (*Cyprinus carpio*) [37], during 8 weeks in African mud catfish (*Clarias gariepinus*) [38], during 175 days in Chinese catfish (*C. fuscus*) [39] and during 12 weeks in Atlantic salmon (*Salmo salar*) [40]. Besides, the performances of triploid fish were not only species and age-dependent but also depended on the experimental conditions and the interactions between the environment and genetics [7]. The individual body size of triploid was more significant due to the larger cell size compared to diploid [41]. However, Aliah et al. [42] reported that the cell size was not correlated with the organ size in sticklebacks (*Gasterosteus aculeatus*). Furthermore, in 2-3 month-old sunshine bass (*Morone* spp.), diploid grew faster compared to triploid [43].

The increase in triploid growth is due to the influence of sterility, diverting energy (nutrient) for somatic growth rather than gonadal development and sexual activity [14]. Most studies concluded that the significant difference in growth rate between triploid and diploid fish occurred during the maturation period in fish such as turbot (*Scophthalmus maximus*) [44] and European sea bass (*Dicentrarchus labrax*) [45]. In this study, it was found that the growth difference (30.0%) between triploid and diploid fish already occurred before (≤ 90-days-old) and during the maturation period (90- to 180-day-old). Also, the growth of triploid showed more significant differences compared to diploid (39.3%). A similar phenomenon has been reported in fancy carp (*C. carpio*) [46].

The role of sexual dimorphism in growth in tilapia has been revealed in the last three decades. Male tilapia grew faster compared to female, so the all-male monosex culture in this species is worldwide applied. Similar cases were found in catfish (*C. gariepinus*) [47] and crucian carp (*Carassius auratus*) [48].

The comparison of the growth performance among the six groups showed that all-male triploid and all-female diploid fish grew faster and lower than the fish in other groups during the experiment. At 120 to 150 days old, the interaction effect between triploidy and sexual dimorphism in growth was not significant among all-female triploid, all-male diploid, and mixed-sex diploid. At 180 days old in the same groups, all-male diploid grew faster than the others, and the interaction effect between triploidy and sexual dimorphism on growth was not significant among all-female triploid and mixed-sex diploid (Figure 2). This phenomenon seemed to be species-specific as found in rainbow trout (*Oncorhynchus mykiss*) by Tabata et al. [49], Mozambique tilapia (*O. mossambicus*) by Varadaraj and Pandian [50] and European sea bass by Felip et al. [51]. Those authors reported that female triploid grew faster than either male triploid, male and female diploid or mixed-sex diploid.

The lowest growth observed in all-female diploid looked as if the female diploid went through rapid reproductive development and sexual maturity. So, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth. In this study, it was recorded that at the age of 120 days old, the majority of female diploid began to spawn and incubate either fertilized or unfertilized eggs in the mouth. This aspect generally allows the female to not feed during eggs incubation for 15 days until larvae can swim freely, as reported by Byamungu et al. [52]. In other words, the role of ploidy level in growth during the maturation period was significantly higher than that before the maturation period. These results also revealed that a high body weight gain in male and female triploid during maturation period seemed to be due to the sterility of triploid fish and reproductive activity of diploid fish.

In this study, triploid fish had higher flesh percentages compared to diploid, and female triploid also had higher flesh percentages. Similar results were reported in gilthead sea bream (*Sparus aurata*) [53] and rainbow trout [54]. However, in common carp [55] up to the size of 400 g, dressing weight of triploid was not significantly different to that of diploid. The results of this study indicated that higher flesh percentages of female triploid compared to male triploid was because the female was more sterile than male, while the higher flesh percentages in triploid compared to diploid seemed correlated with normal in diploid and reducing in triploid through gonadal developments.

Triploid Nile tilapia tends to be high in protein and low in lipid and ash compared to diploid. In terms of sex, male and female fish from both triploid and diploid show the same protein, lipid and carbohydrates contents, while the ash content was significantly different. This result showed that triploidy in Nile tilapia affects flesh quality, especially lipid and ash contents. Further study is needed to gather more valuable information.

The interaction effect between triploidy and sexual dimorphism strongly related to growth had a positive contribution to production performance, especially during the maturation period. Based on the examination of various aspects related to production, the result revealed that all-male triploid Nile tilapia culture has the potential to be developed. Hence, in the future, an applicable method for mass all-male triploid seed production should be considered. One of the possible strategic efforts is how to produce supermale tetraploid as parent stock by combining the chromosome set and hormonal manipulations.

References

1. Devlin R, Biagi CA, Yesaki TY. Growth, viability and genetic characteristics of GH transgenic Coho salmon strains. Aquaculture 2004; 236 (1-4): 607-632. doi: 10.1016/j.aquaculture.2004.02.026
2. Galli L. Genetic modification in aquaculture - a review of potential benefits and risks. Bureau of Rural Sciences, Canberra, Australia. 2002.
3. Pradeep PJ, Srijaya TC, Jose D, Papini A, Hassan A, et al. Identification of diploid and triploid red tilapia by using erythrocyte indices. Caryologia 2011; 64 (4): 485-492. doi: 10.1080/00087114.2011.10589816
4. Lutz CG. Practical genetics for aquaculture. Fishing News Books, Blackwell Science, Oxford. 2001.
5. Felip A, Zanuy S, Carrillo M, Piferrer F. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 2001; 111 (1-3): 175-195.
6. Melamed P, Gong Z, Fletcher G, Hew CL. The potential impact of modern biotechnology on fish aquaculture. Aquaculture 2002; 204 (3-4): 255-269. doi: 10.1016/S0044-8486(01)00838-9
7. Dunham RA. Aquaculture and fisheries biotechnology: genetic approaches. CABI Publishing, Cambridge. 2004.
8. Pradeep PJ, Srijaya TC, Bahuleyan A, Papini A. Can sterility through triploidy induction make an impact on Tilapia industry? International Journal of Aquatic Science 2012; 3 (2): 89-96.
9. Pechsiri J, Yakupitiyage A. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia (*Oreochromis niloticus*

L.). Aquaculture Research 2005; 36 (1): 45-51. doi: 10.1111/j.1365-2109.2004.01182.x

10. Mol K, Byamungu N, Cuisset B, Yaron Z, Ofir M, et al. Hormonal profile of growing male and female diploids and triploids of the blue tilapia (*Oreochromis aureus*) reared in intensive culture. Fish Physiology and Biochemical 1994; 13 (3): 209-218. doi: 10.1007/BF00004359

11. Hussain MG, Rao GPS, Humayun NM, Randall CF, Penman DJ, et al. Comparative performance of growth, biochemical composition and endocrine profiles in diploid and triploid tilapia (*Oreochromis niloticus* L.). Aquaculture 1995; 138 (1-4): 87-97. doi: 10.1016/0044-8486(95)01079-3

12. Puckhaber B, Horstgen-Schwark G. Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). In: Pullin RSV, Lazard M, Legendre JB, Kothlas A, Pauly D (eds): The Third International Symposium on Tilapia in Aquaculture. Manila: ICLARM Conference Proceedings. 1996; pp. 377-382.

13. Bhatta S, Iwai T, Miura T, Huguchi M, Maugars G, et al. Differences between male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). Kathmandu University Journal of Science, Engineering and Technology 2012; 8 (II): 57-65. doi: 10.3126/kuset.v8i2.7326

14. Pradeep PJ, Srijaya TC, Papini A, Chatterji AK. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture 2012; 344-349: 181-187. doi: 10.1016/j.aquaculture.2012.03.006

15. Fuentes-Silva C, Soto-Zarazua GM, Torres-Pacheco I, Flores-Rangel A. Male tilapia production techniques: a mini-review. African Journal of Biotechnology 2013; 12 (36): 5496-5502. doi: 10.5897/AJB11.4119

16. Nguyen CD, David CL. The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in Northern Vietnam. Aquaculture 2000; 184 (3-4): 221-231. doi: 10.1016/S0044-8486(99)00329-4

17. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. Journal of Endocrinology 2001; 170 (1): 27-38. doi: 10.1677/joe.0.1700027

18. Cnaani A, Levavi-Sivan B. Sexual development in fish: practical applications for aquaculture. Sex Development 2009; 3 (2-3): 164-175. doi: 10.1159/000223080

19. Bartley D, Rana K, Immink A. The use of interspecific hybrids in aquaculture and fisheries. Review Fish Biology and Fisheries 2001; 10 (3): 325-337. doi: 10.1023/A:1016691725361

20. Popma TJ, Green BW. Sex reversal of tilapia in earthen ponds. Aquaculture Production Manual, Research and Development Series No. 35, International Center for Aquaculture, Auburn University, Alabama, USA. 1991.

21. Pandian TJ, Sheela SG. Hormonal induction of sex reversal in fish. Aquaculture 1995; 138 (1-4): 1-22. doi: 10.1016/0044-8486(95)01075-0

22. Mukti AT. Optimation of 17α-methyltestosterone synthetic hormone dose and immersion duration in larvae on the success of Nile tilapia (*Oreochromis* sp.) sex reversal. Faculty of Fisheries, Brawijaya University, Malang, Indonesia. 1998.

23. Romerio MP, Fencrich-Verani CSN, Santo De-Copmus BE, Pasilva AS. Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasil Zoology 2000; 29 (3): 654-659. doi: 10.1590/S1516-35982000000300003

24. Mukti AT, Priyambodo B, Rustidja, Widodo MS. Optimization of both 17α-methyltestosterone synthetic hormone dosage and dipping duration of Nile tilapia (*Oreochromis* sp.) larvae on sex reversal efficacy. BIOSAIN Journal of Life Science 2002; 2 (1): 1-8.

25. Mohamed AH, Traifalgar RFM, Serrano Jr. AE, Peralta JP, Pedroso FL. Dietary administration of dehydroepiandrosterone hormone influences the sex differentiation of hybrid red Tilapia (*O. niloticus* × *O. mossambicus*) larvae. Journal of Fisheries and Aquatic Science 2012; 7 (6): 447-453. doi: 10.3923/jfas.2012.44 7.453

26. Beaven U, Muposhi V. Aspects of a monosex population of (*Oreochromis niloticus*) fingerling produced using 17-α methyl testosterone hormone. Journal of Aquaculture Research & Development 2012; 3 (3): 132. doi: 10.4172/2155-9546.1000132

27. Dagne A, Degefu F, Lakew A. Comparative growth performance of monosex and mixed-sex Nile tilapia (*Oreochromis niloticus* L.) in pond culture system at Sebeta, Ethiopian. International Journal of Aquaculture 2013; 3 (7): 30-34. doi: 10.5376/ija.2013.03.0007

28. Ezaz MT, Myers JM, Powell SF, McAndrew BJ, Penman DJ. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia (*Oreochromis*

niloticus L.). Aquaculture 2004; 232 (1-4): 205-214. doi: 10.1016/j.aquaculture.2003.08.001

29. Muller-Belecke A, Horstgen-Schwark G. A YY-male (*Oreochromis niloticus*) strain developed from an exceptional mitotic gynogenetic male and growth performance testing of genetically all-male progenies. Aquaculture Research 2007; 38 (7): 773-775. doi: 10.1111/j.1365-2109.2007.01712.x
30. Aliah RS, Sumantadinata K, Maskur, Naim S. GESIT tilapia: Indonesia's genetic supermales. Global Aquaculture Advocate. May/June. 2010; 36-37.
31. Turra EM, Oliveira DAA, Teixeira EA, Luz RK, Prado SA, et al. Reproduction control in Nile tilapia (*Oreochromis niloticus*) by sexual and chromosome set manipulation. Revista Brasil de Reproduction Animal, Belo Horizonte. 2010; 34 (1): 21-28.
32. Kligerman AD, Bloom SE. Rapid chromosome preparation from solid tissues of fish. Journal of Fisheries Research Board Canada. 1977; 34: 266-269. doi: 10.1139/f77-039
33. Mukti AT, Carman O, Alimuddin, Zairin Jr. M. A rapid chromosome preparation technique without metaphase arrest for ploidy determination in Nile tilapia (*Oreochromis niloticus*). Caryologia 2016; 6 (2): 175-180. doi: 10.1080/00087114.2016.1152112.
34. Buchtova H, Svobodova Z, Kocour M, Velisek J. Evaluation of the dressing percentage of 3-year-old experimental scaly crossbreds of the common carp *Cyprinus carpio* (Linnaeus, 1758) in relation to sex. Acta Veterinary Brno 2006; 75 (1): 123-132. doi: 10.2754/avb200675010123

35. AOAC [Association of Official Analytical Chemists]. Official methods of analysis. (18th eds), Association of Official Analytical Chemists Inc., Washington. 2005.

36. Tave D. Genetics for fish hatchery managers. Avi Publishing, Connecticut. 1993.

37. Mukti AT, Rustidja, Sumitro SB, Djati MS. Polyploidization of common carp (*Cyprinus carpio* L.). BIOSAIN Journal of Life Science 2001; 1 (1): 111-123.

38. Lawson EO, Ishola HA. Effects of cold shock treatment on the survival of fertilized eggs and growth performance of the larvae of African mud catfish *Clarias gariepinus* (Burchell, 1822). Research Journal of Fisheries and Hydrobiology 2010; 5 (2): 85-91.

39. Qin JG, Fast AW, Ako H. Grow-out performance of diploid and triploid Chinese catfish (*Clarias fuscus*). Aquaculture 1998; 166 (3-4): 247-258. doi: 10.1016/S0044-8486(98)00287-7

40. Burke HA, Sacobie CFD, Lall SP, Benfey TJ. The effect of triploidy on juvenile Atlantic salmon (*Salmo salar*) response to varying levels of dietary phosphorus. Aquaculture 2010; 306 (1-4): 295-301. doi: 10.1016/j.aquaculture.2010.05.002

41. Piferrer F, Beaumont A, Falguière J-C, Flajšhans M, Haffray P, et al. Polyploid fish and shellfish: Production, biology, and applications to aquaculture for performance improvement and genetic containment. Aquaculture 2009; 293 (3-4): 125-156. doi: 10.1016/j.aquaculture.2009.04.036

42. Aliah RS, Yamaoka K, Inada Y, Taniguchi N. Effects of triploidy on tissue structure of some organs in ayu. Bulletin Japan Society Science Fisheries 1990; 56 (4): 569-575. doi: 10.2331/suisan.56.569

43. Kerby JH, Eversona JM, Harrell RM, Geiger JG, Starling CC, et al. Performance comparisons between diploid and triploid sunshine bass in freshwater ponds. Aquaculture 2002; 211 (1-4): 91-108. doi: 10.1016/S0044-8486(02)00009-1

44. Cal RM, Vidal S, Gomez C, Ivarez-Blazquez BA, Martinez P, et al. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). Aquaculture 2006; 251 (1): 99-108. doi: 10.1016/j.aquaculture.2005.05.010

45. Felip A, Piferrer F, Zanuy S, Carrillo M. Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons. Journal of Fish Biology 2001; 58 (1): 76-88. doi: 10.1111/j.1095-8649.2001.tb00500.x

46. Taniguchi N, Kijima A, Tamura T, Takegami K, Yamasaki I. Color, growth and maturation in ploidy-manipulated fancy carp. Aquaculture 1986; 57 (1-4): 321-328. doi: 10.1016/0044-8486(86)90210-3

47. Achegbulu CE, Okonji VA, Obi A. Growth and economic performance of diploid and triploid African catfish (*Clarias gariepinus*) in outdoor concrete tanks. International Journal of Genetics 2013; 3 (1): 01-06. doi: 10.5829/idosi.ijg.2013.3.1.738

48. Chen S, Wang J, Liu SJ, Qin QB, Xiao J, et al. Biological characteristics of an improved triploid crucian carp. Science China Series C: Life Science 2009; 52 (8): 733-738. doi: 10.1007/s11427-009-0079-3

49. Tabata YA, Rigolino MG, Tsukamoto RY. Production of all-female triploid rainbow trout (*Oncorhynchus mykiss*) [Pisces, Salmonidae]. III. Growth up to first sexual maturation. Boletim do Instituto de Pesca Sao Paulo 1999; 25: 67-76.

50. Varadaraj K, Pandian TJ. Production of all-female sterile-triploid (*Oreochromis mossambicus*). Aquaculture 1990; 84 (2): 117-123. doi: 10.1016/0044-8486(90)90342-K

51. Felip A, Carrillo M, Zanuy S. Older triploid fish retain impaired reproductive endocrinology in the European sea bass (*Dicentrarchus labrax*). Journal of Fish Biology 2009; 75 (10): 2657-2669. doi: 10.1111/j.1095-8649.2009.02458.x

52. Byamungu N, Darras VM, Kuhn ER. Growth of heat-shock induced triploids of blue tilapia (*Oreochromis aureus*) reared in tanks and in ponds in Eastern Congo: feeding regimes and compensatory growth response of triploid females. Aquaculture 2001; 198 (1-2): 109-122. doi: 10.1016/S0044-8486(00)00605-0

53. Haffray P, Bruant J-S, Facqueur J-M, Fostier A. Gonad development, growth, survival and quality traits in triploids of the protandrous hermaphrodite gilthead sea bream (*Sparus aurata* L.). Aquaculture 2005; 247 (1-4): 107-117. doi: 10.1016/j.aquaculture.2005.02.037

54. Werner C, Poontawee K, Mueller-Belecke A, Horstgen-Schwark G, Wicke M. Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. Archiv Tierzucht 2008; 51 (1): 71-83. doi: 10.5194/aab-51-71-2008

55. Basavaraju Y, Mair GC, Kumar HMM, Kumar SP, Keshavappa GY, et al. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp (Cyprinus carpio) in Karnataka, India. Aquaculture 2002; 204 (3-4): 407-418. doi: 10.1016/S0044-8486(01)00827-4

Table 1. The growth, survival rate, and feed conversion ratio performances of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period (n = 20).

Parameter	Fish group					
	Triploid			Diploid		
	All-male	All-female	Mixed-sex	All-male	All-female	Mixed-sex
Initial biomass (g)	278.6±5.2	190.0±8.3	236.2±6.0	205.0±8.9	136.0±8.8	183.4±5.8
Final biomass (g)	8 056.7±405.5	5 193.3±445.6	7 013.3±551.4	6 130.0±366.6	4 626.7±277.6	5 676.7±465.0
Δ Biomass (g)	7 778.1±404.3^{a}	5 003.0 ±437.9^{e}	6 777.1±548.9^{b}	5 925.0±363.5^{c}	4 490.7±284.9^{f}	5 493.2±462.9^{d}
Δ B 3N - 2N (%)	31.3	11.4	23.4	-	-	-
Initial BW (g)	13.9±0.3	9.5±0.4	11.8±0.3	10.3±0.4	6.8±0.4	9.2±0.3
Final BW (g)	402.8±20.3	278.5±23.2	350.7±27.6	317.0±13.5	252.3±10.2	288.3±15.5
Δ BW (g)	388.9±20.2^{a}	269.0±22.8^{d}	338.9±27.4^{b}	306.7±13.6^{c}	245.5±10.7^{e}	279.2±15.3^{d}
Δ BW 3N - 2 N (%)	26.8	9.6	21.4	-	-	-
Initial BL (mm)	99.2±0.0	93.3±0.0	92.5±0.0	96.3±0.0	92.8±0.0	91.2±0.0
Final BL (mm)	274.5±2.1	241.3±6.7	266.5±5.6	250.0±2.4	232.2±1.9	243.4±4.6
Δ BL (mm)	175.7±2.1^{a}	147.9±6.7^{d}	174.0±5.6^{b}	153.7±2.4^{c}	139.3±1.9^{e}	152.2±4.6^{c}
Δ BL 3N - 2N (%)	14.3	6.2	14.3	-	-	-
AGR (g day^{-1})	3.2±0.2^{a}	2.2±0.2^{d}	2.8±0.2^{b}	2.6±0.1^{c}	2.1±0.1^{e}	2.3±0.1^{d}
Condition factor	1.9±0.1^{b}	2.0±0.0^{bc}	1.9±0.0^{a}	2.0±0.1^{c}	2.0±0.0^{c}	2.0±0.0^{bc}
Feed consumption (g)	470.3±13.8^{c}	367.8±2.0^{b}	378.7±21.5^{b}	380.3±7.0^{b}	341.4±7.4^{a}	378.5±27.3^{b}
Feed conversion ratio	1.2±0.1^{b}	1.4±0.1^{c}	1.1±0.0^{a}	1.2±0.1^{b}	1.4±0.0^{c}	1.4±0.0^{c}
Survival rate (%)	100.0±0.0^{a}	93.3±5.8^{c}	100.0±0.0^{a}	96.7±2.9^{b}	91.7±2.9^{c}	98.3±2.9^{ab}

Note: Δ = gain, Δ B 3N - 2N = relative percentage of triploid:diploid biomass gain, BW = body weight, Δ BW 3N - 2N = relative percentage of triploid:diploid body weight gain, BL = body length, Δ BL 3N - 2N = relative percentage of triploid:diploid body length gain, AGR = absolute growth rate and Feed consumption = amount of given feed. Different superscript in the same row indicates significant differences ($P < 0.05$)

Table 2. Flesh percentages of male and female both triploid and diploid of Nile tilapia fish (n = 10).

Fish group		Body weight (g)	Dressing		Fillet	
			Weight (g)	(%)	Weight (g)	(%)
Triploid	♂	414.1±39.2^{a}	238.3±19.9^{a}	57.6±1.8^{b}	170.9±16.0^{a}	41.3±1.4^{a}
	♀	260.8±24.0^{c}	154.0±13.5^{c}	59.1±1.6^{a}	109.4±10.8^{c}	42.0±1.2^{a}
Diploid	♂	332.0±29.7^{b}	187.2±18.4^{b}	56.4±1.6^{b}	129.4±12.4^{b}	39.0±1.6^{b}
	♀	259.4±14.1^{c}	141.0±7.8^{c}	54.4±1.3^{c}	98.5±6.0^{d}	38.0±1.4^{b}

Note: Different superscript in the same column indicates significant differences ($P < 0.05$)

Table 3. Flesh proximate analysis of male and female both triploid and diploid of Nile tilapia fish (% dry weight) (n = 10).

Fish group		Protein	Lipid	Ash	Carbohydrate
Triploid	♂	85.6 ± 0.3^{ab}	5.1 ± 0.2^{b}	6.2 ± 0.2^{c}	3.2 ± 0.7^{a}
	♀	87.0 ± 1.1^{a}	5.0 ± 0.4^{b}	5.9 ± 0.0^{d}	2.2 ± 1.5^{a}
Diploid	♂	84.2 ± 1.3^{b}	5.9 ± 0.3^{a}	7.1 ± 0.0^{a}	2.8 ± 1.7^{a}
	♀	84.3 ± 1.8^{b}	5.5 ± 0.0^{a}	6.4 ± 0.3^{b}	3.8 ± 1.5^{a}

Note: Different superscript in the same column indicates significant differences ($P < 0.05$)

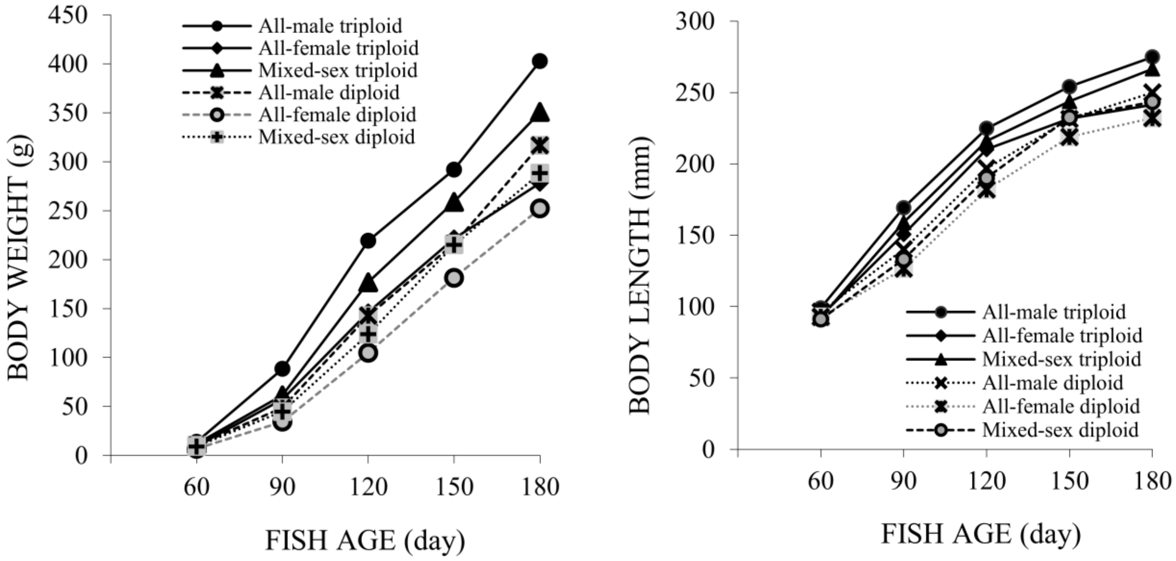

Figure 1. Body weight and body length of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period

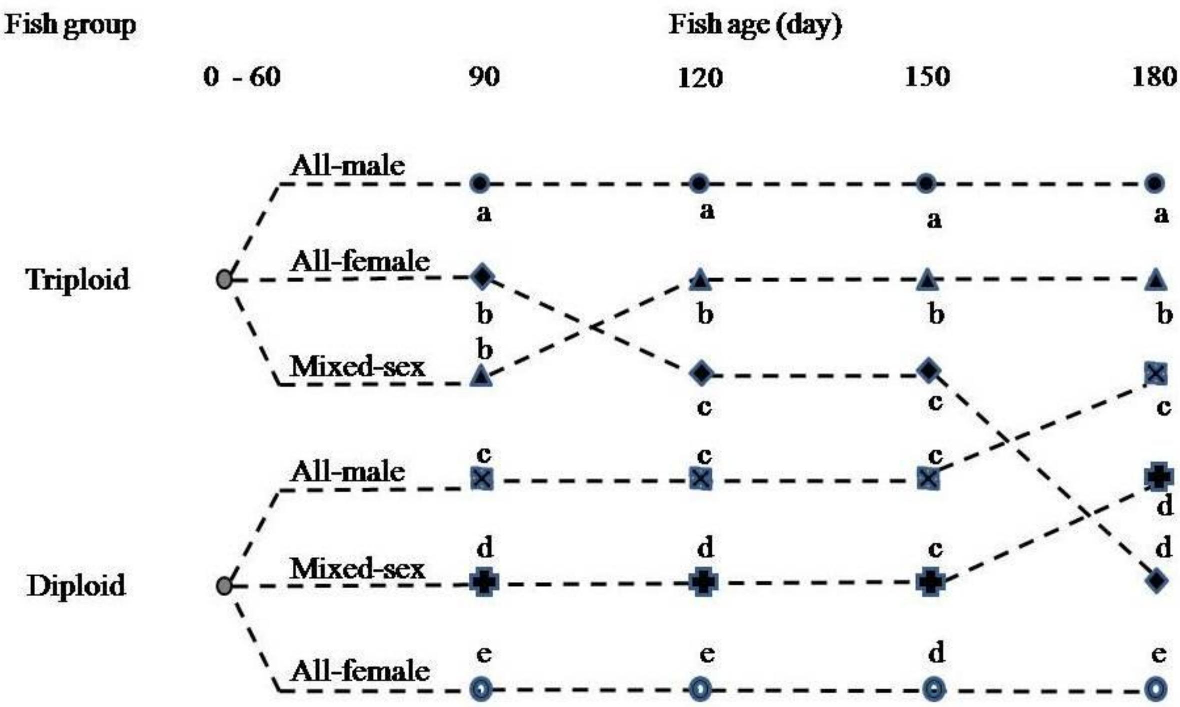

Figure 2. Schematic sequential specific growth rates of triploid and diploid Nile tilapia fish during 4 months grow-out period. Different letter at the same fish age indicates significant differences (P < 0.05)

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Senin, 30 September 2019 13.18 GMT+7

Dear AKHMAD MUKTI,

Please check over the referee evaluation(s) of your manuscript via our online system. You are requested to make corrections in accordance with the evaluation(s) and to respond to any points you disagree with, stating your reasons within 30 days.

Yours sincerely,

Subject Editor

Manuscript Title: Growth performance, survival rate, flesh, and proximate composition of mono- and mixed-sex triploid and diploid Nile tilapia (Oreochromis niloticus)
Manuscript Code Number: VET-1905-79

Web Address: http://online.journals.tubitak.gov.tr

To submit revision, log onto the online system as corresponding author and find your manuscript in the 'Manuscripts Requiring Revision After Reviews' list. Please do not use 'Submit New Manuscript' link to submit your revision.

The details of reviews and the documents uploaded by reviewers can be seen via the online system.
http://online.journals.tubitak.gov.tr

Additional Notes:

Editor's Comments: It is an original research, but the discussion section was insufficient. The manuscript was sent to 3 referees and 2 of them accepted. When the referees' suggestions are completed, the manuscript can be published in the journal.

Reviewers' Comments:

Reviewer1: No any comments

Reviewer2: In this manuscript, it is clearly understood that researchers give an effort to understand growing performance (SGR, Biomass rates, Survival, FCR, Flesh and proximate content) different sex type of diploid and triploid Tilapia. That was an interesting study.
The study is well designed but there are some confusions which were remarked in MS (see attached file please). And some minor changing needs in MS (also remarked in MS).

The MS can be accepted after a minor revision.

Reviewer3: Title of the article was closely related to the experiment and the abstract was almost representative. Results were summarized well enough with figures. On the other hand, the findings of the study were not sufficiently discussed with references and not sufficiently comprehensive in conclusion. Furthermore, there are some gaps in this study;

1. There is no statistical difference between the final weight of triploid females and the final weight of diploid females. The reason remains unexplained. According to the kinds of literature, triploidization lead to increase in sterility and growth. Energy for gamete production is reduced or inhibited. Gonadosomatic indices (GSI) were not nevertheless examined in this study. Therefore, it is not possible to comment on why triploid females have no superiority in terms of growth.
2. There is no homogeneity among groups in terms of initial weights and lengths. In the first two months after hatchings, triploid and diploid genders could reach equal weight by size-specific feeding regime, and then the study could be started without statistical difference among initial weights. There is a mistake in the methodology. As seen in Table 1, possible statistical differences among the initial mean weights of the groups were not analyzed, likewise among the final mean weights. This deficiency was wanted to be skipped because of heterogeneity.
3. What did cause the differences between survival rates of triploid and diploid? What is the advantage of triploidy in terms of survival in Tilapia?
4. Selection of gender in Tilapia can be easily done by manual technique around 20-30 gr weights. So, any fish farmers would not like to culture tilapia with mixing two genders. If triploid female Tilapia are sterile which cause more somatic growth, mix culture of genders are logical and feasible. But, there are no results about sterility and GSI of triploid female, and also whether they reproduce or not in the mixed group according to this study.
Although the study will contribute a little information to the literature, there is a lack of novelty in terms of tilapia triplodization and culture.

Dear
Reviewer 1

Thank you very much for your attention on our article.

Best regards,

Akhmad Taufiq Mukti

Dear
Reviewer 2

Thanks for the corrections and suggestions that have been given to our paper. Authors responses on corrections and suggestions of reviewers have mentioned in the article with blue-colored words or sentences.

1. Reviewer comment: Title; To long and confusing with three "and". Could be shortened.

   Authors response: We have revised the Title of article; page 1, line 1-2: "Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)"

2. Reviewer comment: Abstract; Give this rate with fish/lt or mentioned the volume of aquarium in advance in paranthesis..

   Authors response: We have revised and mentioned word in the Abstract of article; page 1, line 7: "... at a density of 1 fish L^{-1} for 2 months."

3. Reviewer comment: Abstract; If significant used give P value.

   Authors response: We have revised and mentioned word in the Abstract of article; page 1, line 12-17 "($P > 0.05$)."

4. Reviewer comment: Introduction; Change "tests" to "studies".

   Authors response: We have revised and mentioned word in the Abstract of article; page 3, line 3 "... some studies indicated ..."

5. Reviewer comment: Materials and methods; Which strain was used? Or both mixed? Since the study was about growing condition, it should be only one strain GIFT or GET.

   Authors response: In this study, we used the Wanayasa strain of tilapia known as NIRWANA. NIRWANA fish is a strain of tilapia obtained from crossing through a family selection program between GIFT and GET. So it can be interpreted that NIRWANA fish is the offspring of a cross between GIFT and GET.

   We have also mentioned sentences about NIRWANA strain of tilapia in the Materials and methods of article; page 3, line 20-22: "In this study, fish used was the Wanayasa strain of Nile tilapia known as NIRWANA produced through family selection program between the genetic improvement for farmed tilapia (GIFT) and the genetically enhanced tilapia (GET) in Indonesia."

6. Reviewer comment: Materials and methods; Triploid?

   Authors response: Yes triploid Nile tilapia. We have also mentioned sentences in the Materials and methods of article; page 4, line 2-3 "This treatment was produced triploid Nile tilapia of 91-100%, as identified using ..."

7. Reviewer comment: Materials and methods; In total 15+30 days = 45 days (1.5 months) but Figure 1 starts from 60 days (2 months)?.

Authors response: In this study, actually the total age of fish used when starting grow-out using sex group treatment is 60 days (2 months): When larvae aged 2 days, fish were fed of *Moina* for 3 days + tubificid worms for 10 days + commercial feed for 15 days (= 30 days): 2+3+10+15 days = 30 days (1 month), so 30 days (1 month) + 30 days (1 month) = 60 days (2 months).

We have also mentioned sentences in the Materials and methods of article; page 4, line 9-12: "The 2-days-old fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Next, fish were transferred into 180-L aquaria, reared at a density of 4 fish L^{-1} and fed on a commercial diet (40% crude protein content) for 30 days."

8. Reviewer comment: Materials and methods; 2 m x 1 m x 0.7 m dimensions or 1.4 m^3 floating net.

   Authors response: We have revised and mentioned sentences in the Materials and methods of article; page 4, line 22-23 "... reared in 2.0 m × 1.0 m × 0.7 m dimensions of floating net (mesh size of 10 mm) placed in a 20 m × 10 m × 1.5 m dimensions of concrete pond ..."

9. Reviewer comment: Materials and methods; What is the water exchange rate and water quality parameters in ponds? DO, pH, temperature etc?

   Authors response: We have mentioned sentences in the Materials and methods of article; pages 4 and 5, line 24 and line 1-2, respectively: "... with the water exchange rate of 1 L s^{-1}. On the other hand, water qualities, such as temperature, dissolved oxygen, and pH were measured every week with ranges of 27-29 ºC, 3.4-4.4, and 6.7-7.3, respectively."

10. Reviewer comment: Materials and methods; Graphs shows 4 months. And in Results were given by 4 months?

    Authors response: Yes, 4 months: 1 month + 3 months = 4 months (120 days). We have also mentioned sentences in the Materials and methods of article; page 5, line 3-6: "Firstly, fish were fed on a 1-mm-diameter commercial diet (40% crude protein content) ad libitum for 30 days, then they were fed on a 3-mm-diameter commercial diet (33% crude protein content) ad libitum during the last 3 months (90 days), three times a day."

11. Reviewer comment: Materials and methods; In the Table 1, AGR, FCR and K were seen… Please put their formulas in M&M or cite a research you used the formulas from..

    Authors response: We have revised and mentioned sentences in the Materials and methods of article; pages 5 and 6, line 17-22 and line 1, respectively "The formulas were used to calculate absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), respectively, as follows:.."

12. Reviewer comment: Results; Add body weight and length gains.

    Authors response: We have revised and mentioned sentences in the Results of article; page 6, line 10: "The highest values of body weight and length gains ..."

13. Reviewer comment: Results; Need a bit revision it is 21.4 at mixed sex group… this makes confusion…

Authors response: We have mentioned sentences in the Results of article; pages 6, line 11-12: "... mixed-sex triploid (21.4 and 14.3%, respectively),..."

14. Reviewer comment: Results; No need duplication. It would be explained below already.

    Authors response: Thank you for the correction from the reviewer. We have revised and deleted sentences in the Results of article; page 6, line 15.

15. Reviewer comment: Results; In the Table 1, Where is the condition factor? since you give FCR , no need to put Feed consumption in Table. And if you do not use K in results, dont use in Table.

    Authors response: We have revised and deleted parameter of condition factor and feed consumption in the Table 1 of article; page 20.

16. Reviewer comment: Results; Where these come from? Didn't get it? When was the maturation period. If you record kind of data and use t in results put the details in M&M pls.

    Authors response: Figure 1 shows that the difference in body weight between triploid and diploid tilapia, both male and female. The value obtained is a calculation in increasing body weight between male and female triploids and between male and female diploids, both before and during the maturation period, respectively. In general, the maturation period of Nile tilapia occurs after the 90-days-old fish. Therefore, the calculation before maturation period was conducted at the 90^{th} day, while the calculation during maturation period was conducted at the 180^{th} day.

    We have mentioned sentences in the Results of article; page 7, line 3-7: "Before the maturation period, the average body weight of triploid and diploid males was 16.6 and 10.7 g bigger than those of triploid and diploid females, respectively. Meanwhile, during maturation period, the average body weight of triploid and diploid males was 103.3 and 50.5 g bigger than those of triploid and diploid females, respectively."

17. Reviewer comment: Results; Where this came from now? You used AGR in Table 1 and didn't mentioned both in M&M. didn't get what do you mean… seems different than Fig 2. No..intersting of symbol

    Authors response: We have mentioned the formulas, include the specific growth rate (SGR) formula in the Materials and methods of article; pages 5 and 6, line 17-22 and line 1, respectively: "The formulas were used to calculate absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), respectively, as follows:.." and we have mentioned the symbol of SGR in the Figure 2 of article; page 24, line 3: "**Figure 2.** Schematic sequential specific growth rates (SGR) of triploid and diploid ..."

18. Reviewer comment: Results; Didn't get these numbers?? Too much confusing

    Authors response: As well as point 16, the dressing and edible carcass values obtained are the calculation in increasing percentages of dressing and edible carcass between triploid and diploid females and between triploid and diploid males, respectively.

    We have mentioned sentences in the Results of article; pages 7 and 8, line 22-23 and line 1-2, respectively: "The dressing and edible carcass percentages of female triploid were 8.6 and 10.5% higher than those of female diploid, respectively. Meanwhile, the dressing and edible carcass percentages of male triploid were 2.1 and 5.9% higher than those of the diploids, respectively (Table 2).”

19. Reviewer comment: Table 1; Add “s” in group

    Authors response: We have revised and mentioned in the Table 1 of article; page 20: “Fish groups”

20. Reviewer comment: Figure 1; Please us same indicators for same groups in two graphs without grey background.

    Authors response: We have revised about indicator symbol in the Figure 1 of article; page 23.

Thus authors responses on comments, corrections, and suggestions of reviewers, we expect the reviewers and editor were pleased and understand it and we hope that this article will be corrected further. Thank you very much.

Best regards,

Akhmad Taufiq Mukti

Dear
Reviewer 3

Thanks for the corrections and suggestions that have been given to our paper. Authors responses on corrections and suggestions of reviewers have mentioned in the article with blue-colored words or sentences.

1. Reviewer comment: There is no statistical difference between the final weight of triploid females and the final weight of diploid females. The reason remains unexplained. According to the kinds of literature, triploidization lead to increase in sterility and growth. Energy for gamete production is reduced or inhibited. Gonadosomatic indices (GSI) were not nevertheless examined in this study. Therefore, it is not possible to comment on why triploid females have no superiority in terms of growth.

   Authors response: In this study showed that sex is crucial for growth in tilapia. In the Nile tilapia, sex dimorphism determine the speed of fish growth. It is proven that the influence of sex dimorphisms is more dominant than triploidy.

   Based on this study in Table 1 and Figures 1 and 2 show the body weight of female triploid was significantly difference compared to that of female diploid, while increasing body weight of female triploid was no significant difference than mixed-sex diploid because in the mixed-sex group, there are still male sex which has the potential for weight gain on average.

   On the other hand, we have also conducted studies on reproductive performances (gonadal morphology and histology) including gonadosomatic index (GSI) between triploid and diploid Nile tilapia, both male and female. Based on this study, GSI of female triploid has lowest than female diploid. We apologize, the reproductive performances data, including GSI is being submitted for review in other journals.

2. Reviewer comment: There is no homogeneity among groups in terms of initial weights and lengths. In the first two months after hatchings, triploid and diploid genders could reach equal weight by size-specific feeding regime, and then the study could be started without statistical difference among initial weights. There is a mistake in the methodology. As seen in Table 1, possible statistical differences among the initial mean weights of the groups were not analyzed, likewise among the final mean weights. This deficiency was wanted to be skipped because of heterogeneity.

   Authors response: We can not control and homogenize the initial weights and lengths. This is because since the first 2 months of previous rearing at laboratory scale, triploid tilapia has a grow faster and larger (body weight and total length) compared to diploid tilapia, although the triploid and diploid populations are from the same parents and are similar age. This treatment has been repeated 4 times though given the similar type and amount of feed, as we mentioned in the Maaterials and methods of article; page 4, line 7-12.

   Therefore, we was conducted a mean sampling of the initial weight and length of the triploid and diploid fish, both male and female. We was used averages of initial weight and lenght among population. Even, we was used highest average of diploid among population obtained after rearing fry for 2 months at laboratory scale, before being used in this study (field scale). So that, the initial weights and lenghts that we use is a fact that occurs due to the difference in the triploidization treatment in Nile tilapia. We can not control to be homogenous because it might not be homogeneous from the start of the study. We only ensure that we use fish populations of the same age, both triploid and diploid.

3. Reviewer comment: What did cause the differences between survival rates of triploid and diploid? What is the advantage of triploidy in terms of survival in Tilapia?

   Authors response: In this study, survival rate (SR) is likely due to stressed fish after sampling, especially in female diploid which have a high likelihood of maturation, so fish are very susceptible to stress. This may be one of our weaknesses in controlling stress levels and survival of fish after sampling.

4. Reviewer comment: Selection of gender in Tilapia can be easily done by manual technique around 20-30 gr weights. So, any fish farmers would not like to culture tilapia with mixing two genders. If triploid female Tilapia are sterile which cause more somatic growth, mix culture of genders are logical and feasible. But, there are no results about sterility and GSI of triploid female, and also whether they reproduce or not in the mixed group according to this study.

   Authors response: Every month, we always do a total sampling of fish and observe sex through genetalia. During the sampling until the end of this study cleary matches the sex-grouped treatment. During the study, we did not find any all-female or all-male triploids reproducing, including mixed-sex triploid group. Except in diploid tilapia, both all-female and all-male groups are found to undergo maturity and reproduction, including mixed-sex group. This is also as we have mentioned the sentences in the Discussion of article; page 10, line 6-7.

   This is also supported by the observation of GSI and sterility of triploid tilapia, both males and females at the time of monthly sampling, the 3rd month until the 6th month, as the our other studies on the reproductive performances of triploid and diploid Nile tilapia, including GSI and sterility of those male and female fish are being submitted for review in other journals.

5. Reviewer comment: Abstract; gonadosomatic index is one of the most important criterion in triploidy studies of aquaculture. it should had been also investigated.

   Authors response: In the other studies, we were also investigated on reproduction performances, include GSI and sterility of triploid and diploid Nile tilapia, both male and female. However, these parameters would be submitted for review in the process of the other journals.

6. Reviewer comment: Materials and methods; Change “embryos” to “eggs”.

   Authors response: We have changed and mentioned word in the Materials and methods of article; page 3, line 24: “Artificially fertilized eggs ...”

7. Reviewer comment: Materials and methods; Change “closed water recirculation” to “recirculatimg”.

   Authors response: We have changed and mentioned word in the Materials and methods of article; page 4, line 5: “...a recirculating system ...”

8. Reviewer comment: Materials and methods; which weights? in sentences “Sexing was conducted...”

   Authors response: We have mentioned word in the Materials and methods of article; page 4, line 13-14: “... on the average fish weight of 6.5-10 g ...”

9. Reviewer comment: Materials and methods; Delete ”anus, urethra, and”

   Authors response: We have deleted word and mentioned in the Materials and methods of article; page 4, line 13: “.. observing the genital openings ..”.

10. Reviewer comment: Materials and methods; Change “During the first month of the rearing period, the” to “Firstly”.

    Authors response: We have changed and mentioned word in the Materials and methods of article; page 5, line 3: “Firstly, fish were fed ...”

11. Reviewer comment: Materials and methods; Add ad libitum and for 30 days and changed “while the fish” to “then they”.

    Authors response: We have mentioned word in the Materials and methods of article; page 5, line 4: “Firstly, fish were fed ... at satiation for 30 days, then they were fed ...”

    While, we continue to use the term at satiation because in this study, we fed in the fish litle by litle until the fish stop eating (not continuously), so we considered this as feed satiation and not ad libitum. Ad libitum assumed that feed is available at all times continuously.

12. Reviewer comment: Materials and methods; Change “at satiation” to “ad libitum”.

    Authors response: In this study, we continue to use the term at satiation because we fed in the fish litle by litle until the fish stop eating (not continuously), so we considered this as feed satiation and not ad libitum. Ad libitum assumed that feed is available at all times continuously..

13. Reviewer comment: Materials and methods; Change “collected” to “measured”.

    Authors response: We have changed and mentioned word in the Materials and methods of article; page 5, line 8: “... data were measured every month,...”

14. Reviewer comment: Results; Should be checked in terms of english grammar in the sentences “Based on the average body weight before the maturation period, triploid and diploid males had 16.6 and 10.7 g higher than females, respectively. Meanwhile, during the maturation period, triploid and diploid males had 103.3 and 50.5 g bigger than females, respectively.”

    Authors response: We have revised and mentioned the sentences in the Results of article; page 7, line 3-7: “Before the maturation period, the average body weight of triploid and diploid males was 16.6 and 10.7 g bigger than those of triploid and diploid females, respectively. Meanwhile, during maturation period, the average body weight of triploid and diploid males was 103.3 and 50.5 g bigger than those of triploid and diploid females, respectively.”

15. Reviewer comment: Results; Times should be rewritten en d of the sentences as I stated above in the sentences “At 90 days old, all-female and mixed-sex triploids showed the same growth rate. At 120 to 180 days old, the mixed-sex triploid had higher specific growth rate (SGR), while at 120 days old all-female triploid had same SGR as all-male diploid. At 150 days old, all-female triploid, all-male and mixed-sex diploids had same SGR. At 180 days old, all-female triploid had the same SGR as the mixed-sex diploid.”

Authors response: We have revised and mentioned the sentences in the Results of article; page 7, line 11-17: "All-female and mixed-sex triploids groups showed the similar growth rate at the 90th day (Figure 2). The mixed-sex triploid group has higher specific growth rate (SGR) than other sex groups at 120th to 180th day, while all-female triploid group has similar SGR as all-male diploid group at the 120th day. On the other hand, all-female triploid and all-male and mixed-sex diploids groups have similar SGR at the 150th day. Meanwhile, all-female triploid group has similar SGR as the mixed-sex diploid group at the 180th day (Figure 2)."

16. Reviewer comment: Discussion; Delete "during 110 days grow-out period", "during 8 weeks", "during 175 days", and "during 12 weeks".

    Authors response: We have deleted word in the Discussion of article; page 8, line 17-18.

17. Reviewer comment: Discussion; Add "respectively" after "faster and lower"

    Authors response: We have mentioned the word in the Discussion of article; page 9, line 18: "... faster and lower, respectively than ..."

18. Reviewer comment: Discussion; Change "at 120 to 150 days old" to "at 120 to 150th day" and change "at 180 days old" to "at 180th day".

    Authors response: We have mentioned the word in the Discussion of article; page 9, line 21: "...groups at 120th to 150th day." and page 9, line 24: "... diploid at the 180th day (Figure 2)."

19. Reviewer comment: Discussion; "So, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth." To say this, gonadosomatic index should had been investigated.

    Authors response: In the other studies, we were also investigated on reproduction performances, include GSI of triploid and diploid Nile tilapia, both male and female. However, these parameters would be submitted for review in the process of the other journals.

20. Reviewer comment: Discussion; "The results of this study indicated that higher flesh percentages of female triploid compared to male triploid was because the female was more sterile than male, while the higher flesh percentages in triploid compared to diploid seemed correlated with normal in diploid and reducing in triploid through gonadal developments." Not understood. what you mean? how do you know females are more sterile than males? which method did you use for reaching this kind of result?

    Authors response: In the other studies, we was investigated reproduction performances, include gonadal morphological and histologycal of triploid and diploid Nile tilapia, both male and female, including sterility of fish. The study show that the sterility of triploid tilapia, both males and females at the time of monthly sampling, the 3rd month until the 6th month are the fact and clearly. The sterility data, as the our other studies on the reproductive performances of triploid and diploid Nile tilapia are being submitted for review in other journals.

21. Reviewer comment: References; correction of italic "Cyprinus carpio".

    Authors response: We have revised and mentioned in the References of article; page 19, line 7: "... common carp (*Cyprinus carpio*) in ..."

Thus authors responses on comments, corrections, and suggestions of reviewers, we expect the reviewers and editor were pleased and understand it and we hope that this article will be corrected further. Thank you very much.

Best regards,

Akhmad Taufiq Mukti

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Minggu, 20 Oktober 2019 05.02 GMT+7

Dear AKHMAD MUKTI,

The corrected version of your manuscript has not been submitted. In order for us to continue the submission process it must be submitted in the next 10 days.

Manuscript Title: Growth performance, survival rate, flesh, and proximate composition of mono- and mixed-sex triploid and diploid Nile tilapia (Oreochromis niloticus)
Manuscript Code Number: VET-1905-79

Web Address: http://online.journals.tubitak.gov.tr

TUBİTAK Academic Journals Manuscript Submission and Evaluation System

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Senin, 4 November 2019 18.00 GMT+7

Dear AKHMAD MUKTI,

Please check over the referee evaluation(s) of your manuscript via our online system. You are requested to make corrections in accordance with the evaluation(s) and to respond to any points you disagree with, stating your reasons within 30 days.

Yours sincerely,

Subject Editor

Manuscript Title: Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (Oreochromis niloticus)
Manuscript Code Number: VET-1905-79

Web Address: http://online.journals.tubitak.gov.tr

To submit revision, log onto the online system as corresponding author and find your manuscript in the 'Manuscripts Requiring Revision After Reviews' list. Please do not use 'Submit New Manuscript' link to submit your revision.

The details of reviews and the documents uploaded by reviewers can be seen via the online system.
http://online.journals.tubitak.gov.tr

Additional Notes:

Editor's Comments: There are deficiencies especially in the M&M section of the MS. The recommendations/corrections of the referees were not considered sufficient by the authors and were insufficient, careless or incomplete.
The authors did not explain the reasons for the corrections they did not make.
The MS sent back to you to carefully complete the revisions. If the corrections (referees and editors) submitted to you are made carefully, the evaluation of your article will continue.

Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)

Abstract: This study aimed to compare the growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia. The triploid population was obtained through heat shock at 41 ºC for 4 minutes, 4 minutes after fertilization. Before sexing, fish were reared in aquaria at a density of 1 fish L^{-1} for 2 months. After sexing, both triploid and diploid fish were grouped into all-male, all-female, and mixed-sex groups and reared in hapas at a density of 10 fish m^{-2} for 4 months. Each group was replicated three times. The highest body weight, body length, and growth rate were observed in all-male triploid fish, while the lowest values of those parameters were obtained in all-female diploid fish ($P < 0.05$). The highest survival rate was achieved in both all-male and mixed-sex triploids groups ($P < 0.05$) and did not significantly differ from the mixed-sex diploid group ($P > 0.05$). Furthermore, the triploid fish had higher edible carcass percentage compared to diploid. The proximate analysis indicated that the protein content of triploid was higher than that of diploid, while the lipid and ash contents were lower than those of diploid ($P < 0.05$). Triploid Nile tilapia had the best growth performances, including quantity and quality of flesh compared to diploid.

Keywords: Growth performance, triploid production, monosex, mixed-sex, Nile tilapia

1. Introduction

Sterile fish is beneficial in aquaculture because, in the sterile metabolism processes, the fish will reduce or even prevent the use of energy for reproduction. As a result, most of the anabolic energy will be transferred to somatic growth. Sterile fish also have the potential for a better survival rate compared to diploid fish. Devlin et al. [1] stated that the increase in the growth of fish brings substantial benefits in shortening culture period, improving the efficiency of feed utilization and the efficiency of production, and ensuring product availability. Also, culturing sterile fish is one of the best farming management in aquaculture practices, as it enables the use of the metabolism pathway to reach fast somatic tissue instead of producing either sperm or eggs in the spawning season [2].

The high ability (uncontrolled) of tilapia reproduction cause the unexpected density in the pond with varied size and slow growth, making it less commercially profitable in aquaculture. The sterilization is the best possible solution to solve the problems in the tilapia culture [3]. Lutz [4] mentioned that among future's aquaculture commodities, tilapia is a candidate fish to produce functionally sterile seeds on a large scale. The induction of triploidy is one of the methods of producing sterile fish. The culture of triploid fish could provide benefits, such as increased growth, carcass production, survival rate, and flesh quality [5,6,7].

The production of triploid tilapia has been developed for more than four decades, and triploidy is an effective management tool in tilapia farming in the future [8]. Triploid tilapia has small testis or ovaries, low gonad weight and high body weight, protein utilization, and protein efficiency ratio compared to diploid tilapia. Thus,

farming is possibly beneficial [9]. In some cases, the growth performances of triploid tilapia were reported to be superior or equal to those of diploid tilapia [10,11,12].

On the other hand, some studies indicated that male tilapia has faster growth compared to female tilapia [13,14,15]. The production level of monosex male tilapia farming was 10% higher compared to the mixed-sex population [16,17]. Associated with presence of sexual dimorphism in terms of growth, many efforts were made to produce all-male seed population for the purpose of monosex culture, which generally can be obtained through four common methods, namely manual sexing [18] at body size of 5-7 cm, hybridization [7,19], hormonal treatments [15,20,21,22,23,24,25,26,27] or chromosome set manipulations, such as androgenesis [18,28] to produce YY supermale parent stocks [29,30,31].

So far, the combined effects of triploidy and growth-related sexual dimorphism superiorities in tilapia are still unknown. A strain of fish, including tilapia, also possibly influence growth performance during the culture period. Therefore, the present study tries to clarify the effect of those superiorities on growth, survival rate, flesh percentage, and proximate composition of Nile tilapia during the grow-out period.

2. Materials and methods

2.1. Experimental fish preparation

In this study, fish used was the Wanayasa strain of Nile tilapia known as NIRWANA produced through family selection program between the genetic improvement for farmed tilapia (GIFT) and the genetically enhanced tilapia (GET) in Indonesia. The broodstocks were obtained from the Tilapia and Common Carp Aquaculture Development Agency in Purwakarta, West Java, Indonesia. Artificially fertilized eggs

(4 minutes after insemination) were subjected to heat shock treatment at 41 ºC for 4 minutes to produce triploid fish. This treatment was produced triploid Nile tilapia of 91-100%, as identified using the chromosome counting method prepared according to Kligerman and Bloom [32] and Mukti et al. [33]. Embryos were incubated in glass funnel in a recirculating system and diploid fish were produced using a similar procedure.

Larvae of both triploid and diploid were separately reared in 50-L aquaria at a density of 1 fish L^{-1}. A total of 10 aquaria were used for triploid and diploid fish, respectively. The 2-days-old fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Next, fish were transferred into 180-L aquaria, reared at a density of 4 fish L^{-1} and fed on a commercial diet (40% crude protein content) for 30 days. Sexing was conducted morphologically by observing the genital openings on the average fish weight of 6.5-10 g to separate male and female of both triploid and diploid fish. The sexing was also confirmed by gonad preparation and observation using the squash method with acetocarmine stain. Twenty fish from different groups, namely all-male triploid, all-female triploid, mixed-sex triploid, all-male diploid, all-female diploid, and mixed-sex diploid were respectively prepared for performance evaluation.

2.2. Performances evaluation

Previously prepared all-male, all-female, and mixed-sex of both triploid and diploid were separately transferred and reared in 2.0 m × 1.0 m × 0.7 m dimensions of floating net (mesh size of 10 mm) placed in a 20 m × 10 m × 1.5 m dimensions of concrete pond at a density of 10 fish m^{-2} with the water exchange rate of 1 L s^{-1}. On the other hand, water qualities, such as temperature, dissolved oxygen, and pH were measured every week with ranges of 27-29 ºC, 3.4-4.4, and 6.7-7.3, respectively. Three floating nets were used as replication for each group. Firstly, fish were fed on a 1-mm-diameter commercial diet (40% crude protein content) at satiation for 30 days, then they were fed on a 3-mm-diameter commercial diet (33% crude protein content) at satiation during the last 3 months (90 days), three times a day.

The gender of the fish was checked at the monthly sampling time. Body weight, body length, then survival rate and consumed feed data were measured every month, while dressing, edible carcass, and proximate data of male and female both triploid and diploid fish were analyzed at the end of the experiment. The dressing and edible carcass data were determined according to Buchtova et al. [34], and flesh proximate analysis was evaluated according to AOAC [35] based on ten samples from male and female both triploid and diploid, respectively.

2.3. Statistical analysis

Data were statistically analyzed using the analysis of variance (ANOVA) with SPSS ver.10 software. Duncan's multiple range test followed the ANOVA test. The formulas were used to calculate absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), respectively, as follows:

$$\text{AGR (g day}^{-1}) = \frac{\text{Final body weight (g) - Initial body weight (g)}}{\text{Long time of rearing (day)}}$$

$$\text{SGR (\% day}^{-1}) = \frac{\text{Ln final body weight - Ln initial body weight}}{\text{Long time of rearing (day)}} \times 100$$

$$\text{FCR} = \frac{\text{Feed consumed by fish (g)}}{\Delta \text{ Body weight of fish (g)}}$$

$$SR\ (\%) = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

3. Results

3.1. Growth performance, survival rate, and feed conversion ratio

The growth performances of the tested fish groups are shown in Table 1. The results showed that the growth of triploid fish was significantly higher ($P < 0.05$) compared to that of diploid. The biomass gains (Δ B 3N - 2N) of all-male, all-female, and mixed-sex triploids fish were 31.3, 11.4, and 23.4% higher than those of diploid, respectively. A similar pattern was found in body weight gain (Δ BW 3N - 2N) and body length gain (Δ BL 3N - 2N). The highest values of body weight and length gains (26.8 and 14.3%, respectively) were observed in all-male triploid, followed by mixed-sex triploid (21.4 and 14.3%, respectively), while the lowest values (9.6 and 6.2%, respectively) were seen in all-female triploid. Furthermore, all-female diploid fish significantly showed the most inferior growth performance compared to other groups.

All-male triploid had the highest absolute growth rate (AGR) than other groups, followed by mixed-sex triploid, then all-male and all-female diploids. Meanwhile, the mixed-sex triploid had the best feed conversion ratio, followed by all-male triploid and diploid. The survival rates of all-male and mixed-sex triploids and mixed-sex diploid were higher compared to other groups, as shown in Table 1.

Figure 1 shows the monthly body weight and body length recorded during the 4 months grow-out period. In general, triploid grew faster than diploid, and all-male triploid showed the highest growth rate, while all-female diploid showed the lowest growth rate.

In this study, it was observed that in both triploid and diploid fish, males grew faster than females during the experiment. In triploid and diploid groups, the biomass gains of the male were 55.5 and 31.9% higher those of females, respectively. Before the maturation period, the average body weight of triploid and diploid males was 16.6 and 10.7 g bigger than those of triploid and diploid females, respectively. Meanwhile, during maturation period, the average body weight of triploid and diploid males was 103.3 and 50.5 g bigger than those of triploid and diploid females, respectively. These results showed that the role of the sexual dimorphism on growth in Nile tilapia had a similar pattern with the role of the ploidy level, the effects of which were highly significant during the maturation period.

All-female and mixed-sex triploids groups showed the similar growth rate at the 90th day (Figure 2). The mixed-sex triploid group has higher specific growth rate (SGR) than other sex groups at 120th to 180th day, while all-female triploid group has similar SGR as all-male diploid group at the 120th day. On the other hand, all-female triploid and all-male and mixed-sex diploids groups have similar SGR at the 150th day. Meanwhile, all-female triploid group has similar SGR as the mixed-sex diploid group at the 180th day (Figure 2).

3.2. Flesh percentage and proximate composition

The edible carcass percentages of male and female triploids were higher than those of diploids. The highest and lowest dressing percentages were found in triploid and diploid females, respectively ($P < 0.05$). The dressing and edible carcass percentages of female triploid were 8.6 and 10.5% higher than those of female diploid, respectively.

Meanwhile, the dressing and edible carcass percentages of male triploid were 2.1 and 5.9% higher than those of the diploids, respectively (Table 2).

Flesh proximate analysis of triploid and diploid fish is shown in Table 3. The protein content of female triploid was similar to that of male triploid, however was higher than that of diploid fish ($P < 0.05$). On the other hand, lipid and ash contents of male and female triploids were lower than diploids. There were no significant differences in carbohydrate content between triploid and diploid fish.

4. Discussion

This study revealed that ploidy level and sexual dimorphism play essential roles in Nile tilapia growth performance. The high growth of male triploid and low growth of female diploid indicated that both ploidy level and sexual dimorphism significantly affected Nile tilapia growth (Table 1 and Figures 1 and 2).

Tave [36] reported that triploidization leads to increase in sterility and growth. A cell size of triploid is larger than diploid, and energy for gamete production is reduced or inhibited. In most cases, triploid showed heavier body size and faster growth than diploid in common carp (*Cyprinus carpio*) [37], African mud catfish (*Clarias gariepinus*) [38], Chinese catfish (*C. fuscus*) [39], and Atlantic salmon (*Salmo salar*) [40]. Besides, the performances of triploid fish were not only species and age-dependent but also depended on the experimental conditions and the interactions between the environment and genetics [7]. The individual body size of triploid was more significant due to the larger cell size compared to diploid [41]. However, Aliah et al. [42] reported that the cell size was not correlated with the organ size in sticklebacks (*Gasterosteus* *aculeatus*). Furthermore, in 2-3 month-old sunshine bass (*Morone* spp.), diploid grew faster compared to triploid [43].

The increase in triploid growth is due to the influence of sterility, diverting energy (nutrient) for somatic growth rather than gonadal development and sexual activity [14]. Most studies concluded that the significant difference in growth rate between triploid and diploid fish occurred during the maturation period in fish such as turbot (*Scophthalmus maximus*) [44] and European sea bass (*Dicentrarchus labrax*) [45]. In this study, it was found that the growth difference (30.0%) between triploid and diploid fish already occurred before (≤ 90-days-old) and during the maturation period (90- to 180-day-old). Also, the growth of triploid showed more significant differences compared to diploid (39.3%). A similar phenomenon has been reported in fancy carp (*C. carpio*) [46].

The role of sexual dimorphism in growth in tilapia has been revealed in the last three decades. Male tilapia grew faster compared to females, so the all-male monosex culture in this species is worldwide applied. Similar cases were found in catfish (*C. gariepinus*) [47] and crucian carp (*Carassius auratus*) [48].

The comparison of the growth performance among the six groups showed that all-male triploid and all-female diploid fish grew faster and lower, respectively than the fish in other groups during the experiment. The interaction effect between triploidy and sexual dimorphism in growth was not significant among all-female triploid, all-male diploid, and mixed-sex diploid groups at 120th to 150th day. In the same groups, all-male diploid grew faster than the others and the interaction effect between triploidy and sexual dimorphism on growth was not significant among all-female triploid and mixed-sex diploid at the 180th day (Figure 2). This phenomenon seemed to be species-specific as found in rainbow trout (*Oncorhynchus mykiss*) by Tabata et al. [49], Mozambique tilapia (*O. mossambicus*) by Varadaraj and Pandian [50] and European sea bass by Felip et al. [51]. Those authors reported that female triploid grew faster than either male triploid, male and female diploids or mixed-sex diploid.

The lowest growth observed in all-female diploid looked as if the female diploid went through rapid reproductive development and sexual maturity. So, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth. In this study, it was recorded that at the 120th day, the majority of female diploid began to spawn and incubate either fertilized or unfertilized eggs in the mouth. This aspect generally allows the female to not feed during eggs incubation for 15 days until larvae can swim freely, as reported by Byamungu et al. [52]. In other words, the role of the ploidy level in growth during the maturation period was significantly higher than that before the maturation period. These results also revealed that a high body weight gain in male and female triploid during maturation period seemed to be due to the sterility of triploid fish and reproductive activity of diploid fish.

In this study, triploid fish had higher flesh percentages compared to diploid, and female triploid also had higher flesh percentages. Similar results were reported in gilthead sea bream (*Sparus aurata*) [53] and rainbow trout [54]. However, in common carp [55] up to the size of 400 g, the dressing weight of triploid was not significantly different from that of diploid. The results of this study indicated that higher flesh percentages of female triploid compared to male triploid was because the female was more sterile than male, while the higher flesh percentages in triploid compared to diploid seemed correlated with normal in diploid and reducing in triploid through gonadal developments.

Triploid Nile tilapia tends to be high in protein and low in lipid and ash compared to diploid. In terms of sex, male and female fish from both triploid and diploid show the same protein, lipid and carbohydrates contents, while the ash content was significantly different. This result showed that triploidy in Nile tilapia affects flesh quality, especially lipid and ash contents. Further study is needed to gather more valuable information.

The interaction effect between triploidy and sexual dimorphism strongly related to growth had a positive contribution to production performance, especially during the maturation period. Based on the examination of various aspects related to production, the result revealed that all-male triploid Nile tilapia culture has the potential to be developed. Hence, in the future, an applicable method for mass all-male triploid seed production should be considered. One of the possible strategic efforts is how to produce supermale tetraploid as parent stock by combining the chromosome set and hormonal manipulations.

References

1. Devlin R, Biagi CA, Yesaki TY. Growth, viability and genetic characteristics of GH transgenic Coho salmon strains. Aquaculture 2004; 236 (1-4): 607-632. doi: 10.1016/j.aquaculture.2004.02.026
2. Galli L. Genetic modification in aquaculture - a review of potential benefits and risks. Bureau of Rural Sciences, Canberra, Australia. 2002.
3. Pradeep PJ, Srijaya TC, Jose D, Papini A, Hassan A, et al. Identification of diploid and triploid red tilapia by using erythrocyte indices. Caryologia 2011; 64 (4): 485-492. doi: 10.1080/00087114.2011.10589816

4. Lutz CG. Practical genetics for aquaculture. Fishing News Books, Blackwell Science, Oxford. 2001.

5. Felip A, Zanuy S, Carrillo M, Piferrer F. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 2001; 111 (1-3): 175-195.

6. Melamed P, Gong Z, Fletcher G, Hew CL. The potential impact of modern biotechnology on fish aquaculture. Aquaculture 2002; 204 (3-4): 255-269. doi: 10.1016/S0044-8486(01)00838-9

7. Dunham RA. Aquaculture and fisheries biotechnology: genetic approaches. CABI Publishing, Cambridge. 2004.

8. Pradeep PJ, Srijaya TC, Bahuleyan A, Papini A. Can sterility through triploidy induction make an impact on Tilapia industry? International Journal of Aquatic Science 2012; 3 (2): 89-96.

9. Pechsiri J, Yakupitiyage A. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 2005; 36 (1): 45-51. doi: 10.1111/j.1365-2109.2004.01182.x

10. Mol K, Byamungu N, Cuisset B, Yaron Z, Ofir M, et al. Hormonal profile of growing male and female diploids and triploids of the blue tilapia (*Oreochromis aureus*) reared in intensive culture. Fish Physiology and Biochemical 1994; 13 (3): 209-218. doi: 10.1007/BF00004359

11. Hussain MG, Rao GPS, Humayun NM, Randall CF, Penman DJ, et al. Comparative performance of growth, biochemical composition and endocrine profiles in diploid and triploid tilapia (*Oreochromis niloticus* L.). Aquaculture 1995; 138 (1-4): 87-97. doi: 10.1016/0044-8486(95)01079-3

12. Puckhaber B, Horstgen-Schwark G. Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). In: Pullin RSV, Lazard M, Legendre JB, Kothlas A, Pauly D (eds): The Third International Symposium on Tilapia in Aquaculture. Manila: ICLARM Conference Proceedings. 1996; pp. 377-382.

13. Bhatta S, Iwai T, Miura T, Huguchi M, Maugars G, et al. Differences between male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). Kathmandu University Journal of Science, Engineering and Technology 2012; 8 (II): 57-65. doi: 10.3126/kuset.v8i2.7326

14. Pradeep PJ, Srijaya TC, Papini A, Chatterji AK. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture 2012; 344-349: 181-187. doi: 10.1016/j.aquaculture.2012.03.006

15. Fuentes-Silva C, Soto-Zarazua GM, Torres-Pacheco I, Flores-Rangel A. Male tilapia production techniques: a mini-review. African Journal of Biotechnology 2013; 12 (36): 5496-5502. doi: 10.5897/AJB11.4119

16. Nguyen CD, David CL. The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in Northern Vietnam. Aquaculture 2000; 184 (3-4): 221-231. doi: 10.1016/S0044-8486(99)00329-4

17. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. Journal of Endocrinology 2001; 170 (1): 27-38. doi: 10.1677/joe.0.1700027

18. Cnaani A, Levavi-Sivan B. Sexual development in fish: practical applications for aquaculture. Sex Development 2009; 3 (2-3): 164-175. doi: 10.1159/000223080

19. Bartley D, Rana K, Immink A. The use of interspecific hybrids in aquaculture and fisheries. Review Fish Biology and Fisheries 2001; 10 (3): 325-337. doi: 10.1023/A:1016691725361

20. Popma TJ, Green BW. Sex reversal of tilapia in earthen ponds. Aquaculture Production Manual, Research and Development Series No. 35, International Center for Aquaculture, Auburn University, Alabama, USA. 1991.

21. Pandian TJ, Sheela SG. Hormonal induction of sex reversal in fish. Aquaculture 1995; 138 (1-4): 1-22. doi: 10.1016/0044-8486(95)01075-0

22. Mukti AT. Optimation of 17α-methyltestosterone synthetic hormone dose and immersion duration in larvae on the success of Nile tilapia (*Oreochromis* sp.) sex reversal. Faculty of Fisheries, Brawijaya University, Malang, Indonesia. 1998.

23. Romerio MP, Fencrich-Verani CSN, Santo De-Copmus BE, Pasilva AS. Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasil Zoology 2000; 29 (3): 654-659. doi: 10.1590/S1516-35982000000300003

24. Mukti AT, Priyambodo B, Rustidja, Widodo MS. Optimization of both 17α-methyltestosterone synthetic hormone dosage and dipping duration of Nile tilapia (*Oreochromis* sp.) larvae on sex reversal efficacy. BIOSAIN Journal of Life Science 2002; 2 (1): 1-8.

25. Mohamed AH, Traifalgar RFM, Serrano Jr. AE, Peralta JP, Pedroso FL. Dietary administration of dehydroepiandrosterone hormone influences the sex differentiation of hybrid red Tilapia (*O. niloticus* × *O. mossambicus*) larvae. Journal of Fisheries and Aquatic Science 2012; 7 (6): 447-453. doi: 10.3923/jfas.2012.44 7.453

26. Beaven U, Muposhi V. Aspects of a monosex population of (*Oreochromis niloticus*) fingerling produced using 17-α methyl testosterone hormone. Journal of Aquaculture Research & Development 2012; 3 (3): 132. doi: 10.4172/2155-9546.1000132

27. Dagne A, Degefu F, Lakew A. Comparative growth performance of monosex and mixed-sex Nile tilapia (*Oreochromis niloticus* L.) in pond culture system at Sebeta, Ethiopian. International Journal of Aquaculture 2013; 3 (7): 30-34. doi: 10.5376/ija.2013.03.0007

28. Ezaz MT, Myers JM, Powell SF, McAndrew BJ, Penman DJ. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 2004; 232 (1-4): 205-214. doi: 10.1016/j.aquaculture.2003.08.001

29. Muller-Belecke A, Horstgen-Schwark G. A YY-male (*Oreochromis niloticus*) strain developed from an exceptional mitotic gynogenetic male and growth performance testing of genetically all-male progenies. Aquaculture Research 2007; 38 (7): 773-775. doi: 10.1111/j.1365-2109.2007.01712.x

30. Aliah RS, Sumantadinata K, Maskur, Naim S. GESIT tilapia: Indonesia's genetic supermales. Global Aquaculture Advocate. May/June. 2010; 36-37.

31. Turra EM, Oliveira DAA, Teixeira EA, Luz RK, Prado SA, et al. Reproduction control in Nile tilapia (*Oreochromis niloticus*) by sexual and chromosome set manipulation. Revista Brasil de Reproduction Animal, Belo Horizonte. 2010; 34 (1): 21-28.

32. Kligerman AD, Bloom SE. Rapid chromosome preparation from solid tissues of fish. Journal of Fisheries Research Board Canada. 1977; 34: 266-269. doi: 10.1139/f77-039

33. Mukti AT, Carman O, Alimuddin, Zairin Jr. M. A rapid chromosome preparation technique without metaphase arrest for ploidy determination in Nile tilapia (*Oreochromis niloticus*). Caryologia 2016; 6 (2): 175-180. doi: 10.1080/00087114.2016.1152112.

34. Buchtova H, Svobodova Z, Kocour M, Velisek J. Evaluation of the dressing percentage of 3-year-old experimental scaly crossbreds of the common carp *Cyprinus carpio* (Linnaeus, 1758) in relation to sex. Acta Veterinary Brno 2006; 75 (1): 123-132. doi: 10.2754/avb200675010123

35. AOAC [Association of Official Analytical Chemists]. Official methods of analysis. (18th eds), Association of Official Analytical Chemists Inc., Washington. 2005.

36. Tave D. Genetics for fish hatchery managers. Avi Publishing, Connecticut. 1993.

37. Mukti AT, Rustidja, Sumitro SB, Djati MS. Polyploidization of common carp (*Cyprinus carpio* L.). BIOSAIN Journal of Life Science 2001; 1 (1): 111-123.

38. Lawson EO, Ishola HA. Effects of cold shock treatment on the survival of fertilized eggs and growth performance of the larvae of African mud catfish *Clarias gariepinus* (Burchell, 1822). Research Journal of Fisheries and Hydrobiology 2010; 5 (2): 85-91.

39. Qin JG, Fast AW, Ako H. Grow-out performance of diploid and triploid Chinese catfish (*Clarias fuscus*). Aquaculture 1998; 166 (3-4): 247-258. doi: 10.1016/S0044-8486(98)00287-7

40. Burke HA, Sacobie CFD, Lall SP, Benfey TJ. The effect of triploidy on juvenile Atlantic salmon (*Salmo salar*) response to varying levels of dietary phosphorus. Aquaculture 2010; 306 (1-4): 295-301. doi: 10.1016/j.aquaculture.2010.05.002

41. Piferrer F, Beaumont A, Falguière J-C, Flajšhans M, Haffray P, et al. Polyploid fish and shellfish: Production, biology, and applications to aquaculture for performance improvement and genetic containment. Aquaculture 2009; 293 (3-4): 125-156. doi: 10.1016/j.aquaculture.2009.04.036

42. Aliah RS, Yamaoka K, Inada Y, Taniguchi N. Effects of triploidy on tissue structure of some organs in ayu. Bulletin Japan Society Science Fisheries 1990; 56 (4): 569-575. doi: 10.2331/suisan.56.569

43. Kerby JH, Eversona JM, Harrell RM, Geiger JG, Starling CC, et al. Performance comparisons between diploid and triploid sunshine bass in freshwater ponds. Aquaculture 2002; 211 (1-4): 91-108. doi: 10.1016/S0044-8486(02)00009-1

44. Cal RM, Vidal S, Gomez C, Ivarez-Blazquez BA, Martinez P, et al. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). Aquaculture 2006; 251 (1): 99-108. doi: 10.1016/j.aquaculture.2005.05.010

45. Felip A, Piferrer F, Zanuy S, Carrillo M. Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons. Journal of Fish Biology 2001; 58 (1): 76-88. doi: 10.1111/j.1095-8649.2001.tb00500.x

46. Taniguchi N, Kijima A, Tamura T, Takegami K, Yamasaki I. Color, growth and maturation in ploidy-manipulated fancy carp. Aquaculture 1986; 57 (1-4): 321-328. doi: 10.1016/0044-8486(86)90210-3

47. Achegbulu CE, Okonji VA, Obi A. Growth and economic performance of diploid and triploid African catfish (*Clarias gariepinus*) in outdoor concrete tanks. International Journal of Genetics 2013; 3 (1): 01-06. doi: 10.5829/idosi.ijg.2013.3.1.738

48. Chen S, Wang J, Liu SJ, Qin QB, Xiao J, et al. Biological characteristics of an improved triploid crucian carp. Science China Series C: Life Science 2009; 52 (8): 733-738. doi: 10.1007/s11427-009-0079-3

49. Tabata YA, Rigolino MG, Tsukamoto RY. Production of all-female triploid rainbow trout (*Oncorhynchus mykiss*) [Pisces, Salmonidae]. III. Growth up to first sexual maturation. Boletim do Instituto de Pesca Sao Paulo 1999; 25: 67-76.

50. Varadaraj K, Pandian TJ. Production of all-female sterile-triploid (*Oreochromis mossambicus*). Aquaculture 1990; 84 (2): 117-123. doi: 10.1016/0044-8486(90)90342-K

51. Felip A, Carrillo M, Zanuy S. Older triploid fish retain impaired reproductive endocrinology in the European sea bass (*Dicentrarchus labrax*). Journal of Fish Biology 2009; 75 (10): 2657-2669. doi: 10.1111/j.1095-8649.2009.02458.x

52. Byamungu N, Darras VM, Kuhn ER. Growth of heat-shock induced triploids of blue tilapia (*Oreochromis aureus*) reared in tanks and in ponds in Eastern Congo: feeding regimes and compensatory growth response of triploid females. Aquaculture 2001; 198 (1-2): 109-122. doi: 10.1016/S0044-8486(00)00605-0

53. Haffray P, Bruant J-S, Facqueur J-M, Fostier A. Gonad development, growth, survival and quality traits in triploids of the protandrous hermaphrodite gilthead sea bream (*Sparus aurata* L.). Aquaculture 2005; 247 (1-4): 107-117. doi: 10.1016/j.aquaculture.2005.02.037

54. Werner C, Poontawee K, Mueller-Belecke A, Horstgen-Schwark G, Wicke M. Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. Archiv Tierzucht 2008; 51 (1): 71-83. doi: 10.5194/aab-51-71-2008

55. Basavaraju Y, Mair GC, Kumar HMM, Kumar SP, Keshavappa GY, et al. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp (*Cyprinus carpio*) in Karnataka, India. Aquaculture 2002; 204 (3-4): 407-418. doi: 10.1016/S0044-8486(01)00827-4

Table 1. The growth, survival rate, and feed conversion ratio performances of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period (n = 20).

Parameter	Fish groups					
	Triploid			Diploid		
	All-male	All-female	Mixed-sex	All-male	All-female	Mixed-sex
Initial biomass (g)	278.6±5.2	190.0±8.3	236.2±6.0	205.0±8.9	136.0±8.8	183.4±5.8
Final biomass (g)	8 056.7±405.5	5 193.3±445.6	7 013.3±551.4	6 130.0±366.6	4 626.7±277.6	5 676.7±465.0
Δ Biomass (g)	7 778.1±404.3^{a}	5 003.0 ±437.9^{e}	6 777.1±548.9^{b}	5 925.0±363.5^{c}	4 490.7±284.9^{f}	5 493.2±462.9^{d}
Δ B 3N - 2N (%)	31.3	11.4	23.4	-	-	-
Initial BW (g)	13.9±0.3	9.5±0.4	11.8±0.3	10.3±0.4	6.8±0.4	9.2±0.3
Final BW (g)	402.8±20.3	278.5±23.2	350.7±27.6	317.0±13.5	252.3±10.2	288.3±15.5
Δ BW (g)	388.9±20.2^{a}	269.0±22.8^{d}	338.9±27.4^{b}	306.7±13.6^{c}	245.5±10.7^{e}	279.2±15.3^{d}
Δ BW 3N - 2 N (%)	26.8	9.6	21.4	-	-	-
Initial BL (mm)	99.2±0.0	93.3±0.0	92.5±0.0	96.3±0.0	92.8±0.0	91.2±0.0
Final BL (mm)	274.5±2.1	241.3±6.7	266.5±5.6	250.0±2.4	232.2±1.9	243.4±4.6
Δ BL (mm)	175.7±2.1^{a}	147.9±6.7^{d}	174.0±5.6^{b}	153.7±2.4^{c}	139.3±1.9^{e}	152.2±4.6^{c}
Δ BL 3N - 2N (%)	14.3	6.2	14.3	-	-	-
AGR (g day^{-1})	3.2±0.2^{a}	2.2±0.2^{d}	2.8±0.2^{b}	2.6±0.1^{c}	2.1±0.1^{e}	2.3±0.1^{d}
Feed conversion ratio	1.2±0.1^{b}	1.4±0.1^{c}	1.1±0.0^{a}	1.2±0.1^{b}	1.4±0.0^{c}	1.4±0.0^{c}
Survival rate (%)	100.0±0.0^{a}	93.3±5.8^{c}	100.0±0.0^{a}	96.7±2.9^{b}	91.7±2.9^{c}	98.3±2.9^{ab}

Note: Δ = gain, Δ B 3N - 2N = relative percentage of triploid:diploid biomass gain, BW = body weight, Δ BW 3N - 2N = relative percentage of triploid:diploid body weight gain, BL = body length, Δ BL 3N - 2N = relative percentage of triploid:diploid body length gain, and AGR = absolute growth rate. Different superscripts in the same row indicates significant differences ($P < 0.05$)

Table 2. Flesh percentages of male and female both triploid and diploid of Nile tilapia fish (n = 10).

Fish group		Body weight (g)	Dressing		Edible carcass	
			Weight (g)	(%)	Weight (g)	(%)
Triploid	♂	414.1±39.2[a]	238.3±19.9[a]	57.6±1.8[b]	170.9±16.0[a]	41.3±1.4[a]
	♀	260.8±24.0[c]	154.0±13.5[c]	59.1±1.6[a]	109.4±10.8[c]	42.0±1.2[a]
Diploid	♂	332.0±29.7[b]	187.2±18.4[b]	56.4±1.6[b]	129.4±12.4[b]	39.0±1.6[b]
	♀	259.4±14.1[c]	141.0±7.8[c]	54.4±1.3[c]	98.5±6.0[d]	38.0±1.4[b]

Note: Different superscripts in the same column indicates significant differences (P < 0.05)

Table 3. Flesh proximate analysis of male and female both triploid and diploid of Nile tilapia fish (% dry weight) (n = 10).

Fish group		Protein	Lipid	Ash	Carbohydrate
Triploid	♂	85.6±0.3^{ab}	5.1±0.2^{b}	6.2±0.2^{c}	3.2±0.7^{a}
	♀	87.0±1.1^{a}	5.0±0.4^{b}	5.9±0.0^{d}	2.2±1.5^{a}
Diploid	♂	84.2±1.3^{b}	5.9±0.3^{a}	7.1±0.0^{a}	2.8±1.7^{a}
	♀	84.3±1.8^{b}	5.5±0.0^{a}	6.4±0.3^{b}	3.8±1.5^{a}

Note: Different superscripts in the same column indicates significant differences ($P < 0.05$)

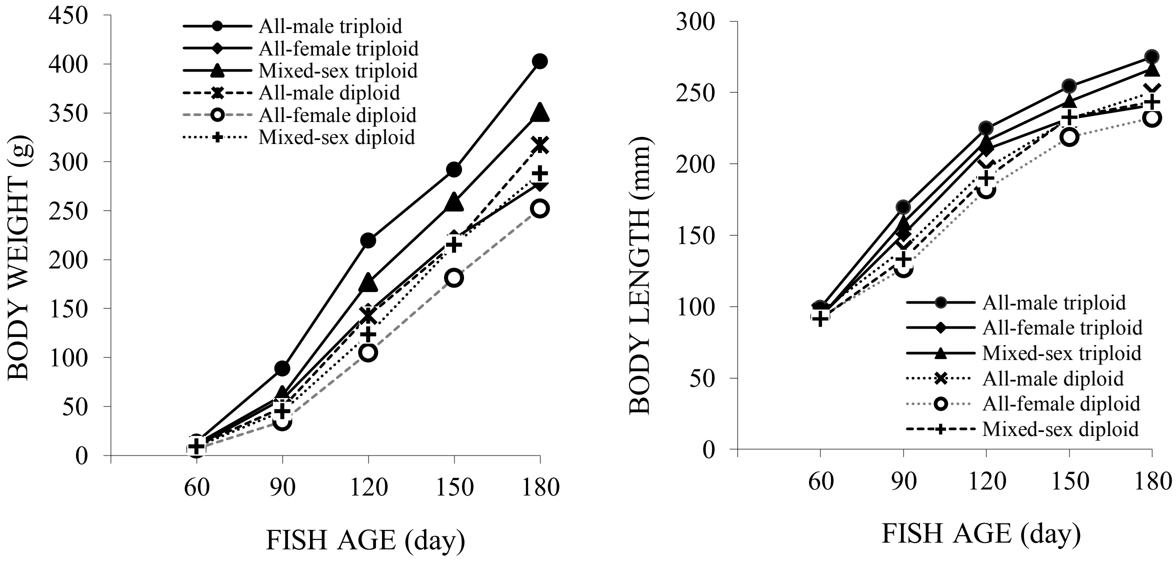

Figure 1. Body weight and body length of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period

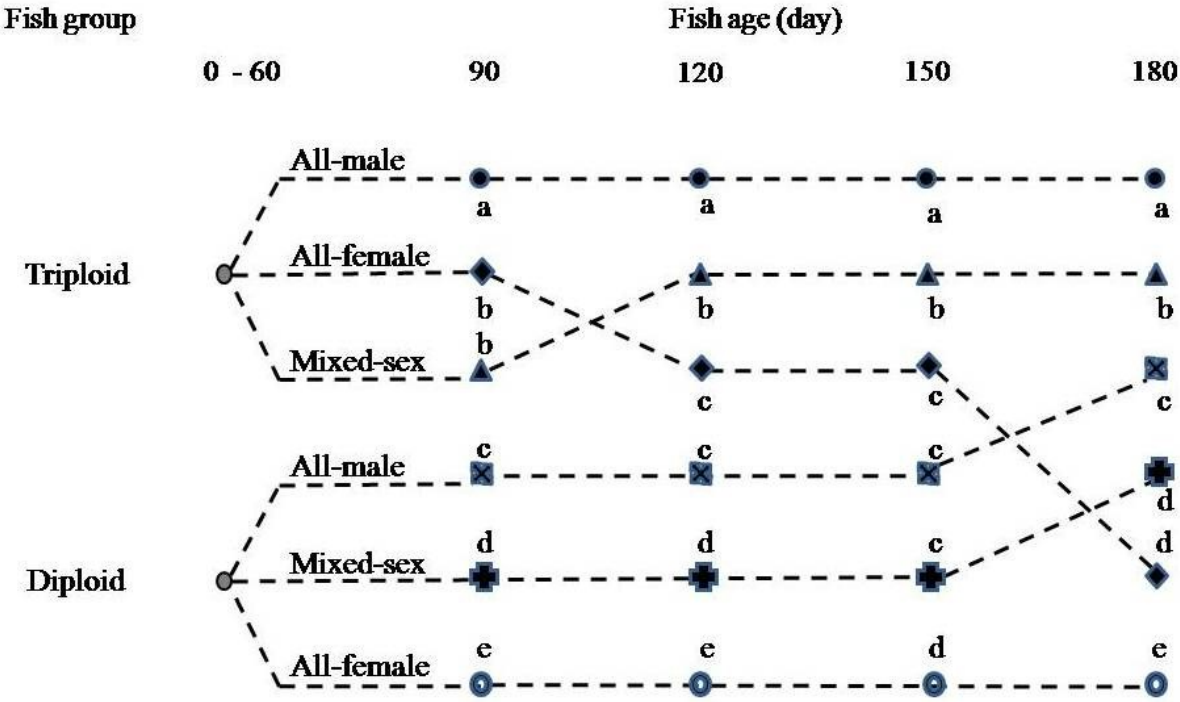

Figure 2. Schematic sequential specific growth rate (SGR) of triploid and diploid Nile tilapia fish during 4 months grow-out period. Different letters at the same fish age indicates significant differences ($P < 0.05$)

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Minggu, 24 November 2019 06.00 GMT+7

Dear AKHMAD MUKTI,

The corrected version of your manuscript has not been submitted. In order for us to continue the submission process it must be submitted in the next 10 days.

Manuscript Title: Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (Oreochromis niloticus)
Manuscript Code Number: VET-1905-79

Web Address: http://online.journals.tubitak.gov.tr

TUBİTAK Academic Journals Manuscript Submission and Evaluation System

Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)

Abstract: This study aimed to compare the growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia. The triploid population was obtained through heat shock at 41 ºC for 4 minutes, 4 minutes after fertilization. Before sexing, 50 fish were reared in aquaria at a density of 1 fish L^{-1} for 2 months. After sexing, both triploid and diploid fish were grouped into all-male, all-female, and mixed-sex groups and reared in hapas at a density of 10 fish m^{-2} for 4 months. Each group was replicated three times. The highest body weight, body length, and growth rate were observed in all-male triploid fish, while the lowest values of those parameters were obtained in all-female diploid fish ($P < 0.05$). The highest survival rate was achieved in both all-male and mixed-sex triploids groups ($P < 0.05$) and did not significantly differ from the mixed-sex diploid group ($P > 0.05$). Furthermore, the triploid fish had higher edible carcass percentage compared to diploid. The proximate analysis indicated that the protein content of triploid was higher than that of diploid, while the lipid and ash contents were lower than those of diploid ($P < 0.05$). Triploid Nile tilapia had the best growth performances, including quantity and quality of flesh compared to diploid.

Formatted: English (United States)

Keywords: Growth performance, triploid production, monosex, mixed-sex, Nile tilapia

1. Introduction

Sterile fish is beneficial in aquaculture because, in the sterile metabolism processes, the fish will reduce or even prevent the use of energy for reproduction. As a result, most of the anabolic energy will be transferred to somatic growth. Sterile fish also have the potential for a better survival rate compared to diploid fish. Devlin et al. [1] stated that the increase in the growth of fish brings substantial benefits in shortening culture period, improving the efficiency of feed utilization and the efficiency of production, and ensuring product availability. Also, culturing sterile fish is one of the best farming management in aquaculture practices, as it enables the use of the metabolism pathway to reach fast somatic tissue instead of producing either sperm or eggs in the spawning season [2].

The high ability (uncontrolled) of tilapia reproduction causes the unexpected density in the pond with varied size and slow growth, making it less commercially profitable in aquaculture. The sterilization is the best possible solution to solve the problems in the tilapia culture [3]. Lutz [4] mentioned that among future's aquaculture commodities, tilapia is a candidate fish to produce functionally sterile seeds on a large scale. The induction of triploidy is one of the methods of producing sterile fish. The culture of triploid fish could provide benefits, such as increased growth, carcass production, survival rate, and flesh quality [5,6,7].

The production of triploid tilapia has been developed for more than four decades, and triploidy is an effective management tool in tilapia farming in the future [8]. Triploid tilapia has small testis or ovaries, low gonad weight and high body weight, protein utilization, and protein efficiency ratio compared to diploid tilapia. Thus,

farming is possibly beneficial [9]. In some cases, the growth performances of triploid tilapia were reported to be superior or equal to those of diploid tilapia [10,11,12].

On the other hand, some studies indicated that male tilapia has faster growth compared to female tilapia [13,14,15]. The production level of monosex male tilapia farming was 10% higher compared to the mixed-sex population [16,17]. Associated with presence of sexual dimorphism in terms of growth, many efforts were made to produce all-male seed population for the purpose of monosex culture, which generally can be obtained through four common methods, namely manual sexing [18] at body size of 5-7 cm, hybridization [7,19], hormonal treatments [15,20,21,22,23,24,25,26,27] or chromosome set manipulations, such as androgenesis [18,28] to produce YY supermale parent stocks [29,30,31].

So far, the combined effects of triploidy and growth-related sexual dimorphism superiorities in tilapia are still unknown. A strain of fish, including tilapia, also possibly influence growth performance during the culture period. Therefore, the present study tries to clarify the effect of those superiorities on growth, survival rate, flesh percentage, and proximate composition of Nile tilapia during the grow-out period.

2. Materials and methods

2.1. Experimental fish preparation

In this study, fish used was the Wanayasa strain of Nile tilapia known as NIRWANA produced through family selection program between the genetic improvement for farmed tilapia (GIFT) and the genetically enhanced tilapia (GET) in Indonesia. The broodstocks were obtained from the Tilapia and Common Carp Aquaculture Development Agency in Purwakarta, West Java, Indonesia. Artificially fertilized eggs

(4 minutes after insemination) were subjected to heat shock treatment at 41 ºC for 4 minutes to produce triploid fish. This treatment was produced triploid Nile tilapia of 91-100%, as identified using the chromosome counting method prepared according to Kligerman and Bloom [32] and Mukti et al. [33]. Embryos were incubated in glass funnel in a recirculating system and diploid fish were produced using a similar procedure.

Larvae of both triploid and diploid were separately reared in 50-L aquaria at a density of 1 fish L^{-1}. A total of 10 aquaria were used for triploid and diploid fish, respectively. The 2-days-old fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Next, fish were transferred into 180-L aquaria, reared at a density of 4 fish L^{-1} and fed on a commercial diet (40% crude protein content) for 30 days. Sexing was conducted morphologically by observing the genital openings on the average fish weight of 6.5-10 g to separate male and female of both triploid and diploid fish. The sexing was also confirmed by gonad preparation and observation using the squash method with acetocarmine stain. Twenty fish from different groups, namely all-male triploid, all-female triploid, mixed-sex triploid, all-male diploid, all-female diploid, and mixed-sex diploid were respectively prepared for performance evaluation.

2.2. Performances evaluation

Previously prepared all-male, all-female, and mixed-sex of both triploid and diploid were separately transferred and reared in 2.0 m × 1.0 m × 0.7 m dimensions of floating net (mesh size of 10 mm) placed in a 20 m × 10 m × 1.5 m dimensions of concrete pond at a density of 10 fish m^{-2} with the water exchange rate of 1 L s^{-1}. On the other hand, water qualities, such as temperature, dissolved oxygen, and pH were measured every week with ranges of 27-29 ºC, 3.4-4.4 mg L^{-1}, and 6.7-7.3, respectively. Three floating nets were used as replication for each group. Firstly, fish were fed on a 1-mm-diameter commercial diet (40% crude protein content) at satiation for 30 days, then they were fed on a 3-mm-diameter commercial diet (33% crude protein content) at satiation during the last 3 months (90 days), three times a day.

~~The~~ gender of the fish was checked ~~at the~~ monthly~~ sampling tim~~e. Body weight, body length, then survival rate and ~~consumed~~ feed intake ~~data~~ were measured every month, while dressing, edible carcass, and proximate data of male and female both triploid and diploid fish were analyzed at the end of the experimental period.

The formulas were used to calculate absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), respectively, as follows:

$$\text{AGR (g day}^{-1}) = \frac{\text{Final body weight (g) - Initial body weight (g)}}{\text{Long time of rearing (day)}}$$

$$\text{SGR (\% day}^{-1}) = \frac{\text{Ln final body weight - Ln initial body weight}}{\text{Long time of rearing (day)}} \times 100$$

$$\text{FCR} = \frac{\text{Feed consumed by fish (g)}}{\Delta \text{ Body weight of fish (g)}}$$

$$\text{SR (\%)} = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

The dressing and edible carcass data were determined according to protocol of Buchtova et al. [34]~~,~~. The dressing and edible carcass were calculated by using formula, respectively:

$$\text{Dressing (\%)} = \frac{\text{Dressing weight of fish (g)}}{\text{Body weight of fish (g)}} \times 100$$

Formatted: Font: English (United States)

Formatted: Font: English (United States)

Formatted: Indent: First line: 0.3"

Commented [WK1]: ???

Commented [WK2]: How was it calculated?

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Formatted: Indent: First line: 0.3"

Field Code Changed

$$\text{Edible carcass (\%)} = \frac{\text{Edible carcass weight of fish (g)}}{\text{Body weight of fish (g)}} \times 100$$

In addtion, and flesh proximate analysis of fish (crude protein, crude lipid, ash, and carbohidrate contents) was evaluated according to AOAC protocol [35] based on ten samples from male and female both triploid and diploid, respectively.

The formulas were used to calculate absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), respectively, as follows:

$$\text{AGR (g day}^{-1}) = \frac{\text{Final body weight (g) - Initial body weight (g)}}{\text{Long time of rearing (day)}}$$

$$\text{SGR (\% day}^{-1}) = \frac{\text{Ln final body weight - Ln initial body weight}}{\text{Long time of rearing (day)}} \times 100$$

$$\text{FCR} = \frac{\text{Feed consumed by fish (g)}}{\Delta \text{ Body weight of fish (g)}}$$

$$\text{SR (\%)} = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

2.3. Statistical analysis

Data were statistically analyzed using the analysis of variance (ANOVA) with SPSS ver.10 software. Duncan's multiple range test followed the ANOVA test.

The formulas were used to calculate absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), respectively, as follows:

$$\text{AGR (g day}^{-1}) = \frac{\text{Final body weight (g) - Initial body weight (g)}}{\text{Long time of rearing (day)}}$$

$$\text{SGR (\% day}^{-1}) = \frac{\text{Ln final body weight - Ln initial body weight}}{\text{Long time of rearing (day)}} \times 100$$

Field Code Changed

Commented [WK3]: Need more explanation.

Formatted: Indent: First line: 0.3"

Formatted: Font color: Auto, Indonesian

Commented [WK4]: Not right place. These formulas must be given before Statistical analysis in the right place. Please give references

Formatted: Indonesian

Commented [WK5]: Need more detail

Formatted: English (United States)

Formatted: HTML Preformatted, Space After: 0 pt, Line spacing: Double

Formatted: English (United States)

Formatted: English (United States)

Formatted: English (United States)

Formatted: HTML Preformatted, Space Before: 0 pt, After: 0 pt, Line spacing: Double

Formatted: English (United States)

Formatted: English (United States)

$$\text{FCR} = \frac{\text{Feed consumed by fish (g)}}{\Delta\ \text{Body weight of fish (g)}}$$

$$\text{SR (\%)} = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

3. Results

3.1. Growth performance, survival rate, and feed conversion ratio

The growth performances of the tested fish groups are shown in Table 1. The results showed that the growth of triploid fish was significantly higher ($P < 0.05$) compared to that of diploid. The biomass gains (Δ B 3N - 2N) of all-male, all-female, and mixed-sex triploids fish were 31.3, 11.4, and 23.4% higher than those of diploid, respectively. A similar pattern was found in body weight gain (Δ BW 3N - 2N) and body length gain (Δ BL 3N - 2N). The highest values of body weight and length gains (26.8 and 14.3%, respectively) were observed in all-male triploid, followed by mixed-sex triploid (21.4 and 14.3%, respectively), while the lowest values (9.6 and 6.2%, respectively) were seen in all-female triploid. Furthermore, all-female diploid fish significantly showed the most inferior growth performance compared to other groups.

All-male triploid had the highest absolute growth rate (AGR) than other groups, followed by mixed-sex triploid, then all-male and all-female diploids. Meanwhile, the mixed-sex triploid had the best feed conversion ratio, followed by all-male triploid and diploid. The survival rates of all-male and mixed-sex triploids and mixed-sex diploid were higher compared to other groups, as shown in Table 1.

Figure 1 shows the monthly body weight and body length recorded during the 4 months grow-out period. In general, triploid grew faster than diploid, and all-male

Commented [WK6]: Not right place. These formulas must be given before Statistical analysis in the right place. Please give references

Formatted: Font: English (United States)

Formatted: Tab stops: 1.27", Left + Not at 0.98"

Formatted: Font: English (United States)

Formatted: Justified

triploid showed the highest growth rate, while all-female diploid showed the lowest growth rate.

In this study, it was observed that in both triploid and diploid fish, males grew faster than females during the experiment. In triploid and diploid groups, the biomass gains of the male were 55.5 and 31.9% higher those of females, respectively. Before the maturation period, the average body weight of triploid and diploid males was 16.6 and 10.7 g bigger than those of triploid and diploid females, respectively. Meanwhile, during maturation period, the average body weight of triploid and diploid males was 103.3 and 50.5 g bigger than those of triploid and diploid females, respectively. These results showed that the role of the sexual dimorphism on growth in Nile tilapia had a similar pattern with the role of the ploidy level, the effects of which were highly significant during the maturation period.

Commented [WK7]: ?????no information in M&M. You have not any measurement.

All-female and mixed-sex triploids groups showed the similar growth rate at the 90th day (Figure 2). The mixed-sex triploid group has higher specific growth rate (SGR) than other sex groups at 120th to 180th day, while all-female triploid group has similar SGR as all-male diploid group at the 120th day. On the other hand, all-female triploid and all-male and mixed-sex diploids groups have similar SGR at the 150th day. Meanwhile, all-female triploid group has similar SGR as the mixed-sex diploid group at the 180th day (Figure 2).

3.2. Flesh percentage and proximate composition

The edible carcass percentages of male and female triploids were higher than those of diploids. The highest and lowest dressing percentages were found in triploid and diploid females, respectively ($P < 0.05$). The dressing and edible carcass percentages of female triploid were 8.6 and 10.5% higher than those of female diploid, respectively. Meanwhile, the dressing and edible carcass percentages of male triploid were 2.1 and 5.9% higher than those of the diploids, respectively (Table 2).

Commented [WK8]: ???? Table 2 show different percentage results.

Flesh proximate analysis of triploid and diploid fish is shown in Table 3. The protein content of female triploid was similar to that of male triploid, however was higher than that of diploid fish ($P < 0.05$). On the other hand, lipid and ash contents of male and female triploids were lower than diploids. There were no significant differences in carbohydrate content between triploid and diploid fish.

4. Discussion

This study revealed that ploidy level and sexual dimorphism play essential roles in Nile tilapia growth performance. The high growth of male triploid and low growth of female diploid indicated that both ploidy level and sexual dimorphism significantly affected Nile tilapia growth (Table 1 and Figures 1 and 2).

Tave [36] reported that triploidization leads to increase in sterility and growth. A cell size of triploid is larger than diploid, and energy for gamete production is reduced or inhibited. In most cases, triploid showed heavier body size and faster growth than diploid in common carp (*Cyprinus carpio*) [37], African mud catfish (*Clarias gariepinus*) [38], Chinese catfish (*C. fuscus*) [39], and Atlantic salmon (*Salmo salar*) [40]. Besides, the performances of triploid fish were not only species and age-dependent but also depended on the experimental conditions and the interactions between the environment and genetics [7]. The individual body size of triploid was more significant due to the larger cell size compared to diploid [41]. However, Aliah et al. [42] reported that the cell size was not correlated with the organ size in sticklebacks (*Gasterosteus* *aculeatus*). Furthermore, in 2-3 month-old sunshine bass (*Morone* spp.), diploid grew faster compared to triploid [43].

The increase in triploid growth is due to the influence of sterility, diverting energy (nutrient) for somatic growth rather than gonadal development and sexual activity [14]. Most studies concluded that the significant difference in growth rate between triploid and diploid fish occurred during the maturation period in fish such as turbot (*Scophthalmus maximus*) [44] and European sea bass (*Dicentrarchus labrax*) [45]. In this study, it was found that the growth difference (30.0%) between triploid and diploid fish already occurred before (≤ 90-days-old) and during the maturation period (90- to 180-day-old). Also, the growth of triploid showed more significant differences compared to diploid (39.3%). A similar phenomenon has been reported in fancy carp (*C. carpio*) [46].

The role of sexual dimorphism in growth in tilapia has been revealed in the last three decades. Male tilapia grew faster compared to females, so the all-male monosex culture in this species is worldwide applied. Similar cases were found in catfish (*C. gariepinus*) [47] and crucian carp (*Carassius auratus*) [48].

The comparison of the growth performance among the six groups showed that all-male triploid and all-female diploid fish grew faster and lower, respectively than the fish in other groups during the experiment. The interaction effect between triploidy and sexual dimorphism in growth was not significant among all-female triploid, all-male diploid, and mixed-sex diploid groups at 120th to 150th day. In the same groups, all-male diploid grew faster than the others and the interaction effect between triploidy and sexual dimorphism on growth was not significant among all-female triploid and mixed-sex diploid at the 180th day (Figure 2). This phenomenon seemed to be species-specific

Formatted: Font: English (United States) as found in rainbow trout (*Oncorhynchus mykiss*) by Tabata et al. [49], Mozambique tilapia (*O. mossambicus*) by Varadaraj and Pandian [50] and European sea bass by Felip et al. [51]. Those authors reported that female triploid grew faster than either male triploid, male and female diploids or mixed-sex diploid.

The lowest growth was observed in all-female diploid looked as if the female diploid went through rapid reproductive development and sexual maturity. So, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth. In this study, it was recorded that at the 120th day, the majority of female diploid began to spawn and incubate either fertilized or unfertilized eggs in the mouth. This aspect generally allows the female to not feed during eggs incubation for 15 days until larvae can swim freely, as reported by Byamungu et al. [52]. In other words, the role of the ploidy level in growth during the maturation period was significantly higher than that before the maturation period. These results also revealed that a high body weight gain in male and female triploid during maturation period seemed to be due to the sterility of triploid fish and reproductive activity of diploid fish.

In this study, triploid fish had higher flesh percentages compared to diploid, and female triploid also had higher flesh percentages. Similar results were reported in gilthead sea bream (*Sparus aurata*) [53] and rainbow trout [54]. However, in common carp [55] up to the size of 400 g, the dressing weight of triploid was not significantly different from that of diploid. The results of this study indicated that higher flesh percentages of female triploid compared to male triploid was because the female was more sterile than male, while the higher flesh percentages in triploid compared to diploid seemed correlated with normal in diploid and reducing in triploid through gonadal developments.

Formatted: English (United States)

Triploid Nile tilapia tends to be high in protein and low in lipid and ash compared to diploid. In terms of sex, male and female fish from both triploid and diploid show the same protein, lipid and carbohydrates contents, while the ash content was significantly different. This result showed that triploidy in Nile tilapia affects flesh quality, especially lipid and ash contents. Further study is needed to gather more valuable information.

Commented [WK9]: Discussion with other author's result.

The interaction effect between triploidy and sexual dimorphism strongly related to growth had a positive contribution to production performance, especially during the maturation period. Based on the examination of various aspects related to production, the result revealed that all-male triploid Nile tilapia culture has the potential to be developed. Hence, in the future, an applicable method for mass all-male triploid seed production should be considered. One of the possible strategic efforts is how to produce supermale tetraploid as parent stock by combining the chromosome set and hormonal manipulations.

References

1. Devlin R, Biagi CA, Yesaki TY. Growth, viability and genetic characteristics of GH transgenic Coho salmon strains. Aquaculture 2004; 236 (1-4): 607-632. doi: 10.1016/j.aquaculture.2004.02.026
2. Galli L. Genetic modification in aquaculture - a review of potential benefits and risks. Bureau of Rural Sciences, Canberra, Australia. 2002.
3. Pradeep PJ, Srijaya TC, Jose D, Papini A, Hassan A, et al. Identification of diploid and triploid red tilapia by using erythrocyte indices. Caryologia 2011; 64 (4): 485-492. doi: 10.1080/00087114.2011.10589816

4. Lutz CG. Practical genetics for aquaculture. Fishing News Books, Blackwell Science, Oxford. 2001.

5. Felip A, Zanuy S, Carrillo M, Piferrer F. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 2001; 111 (1-3): 175-195.

6. Melamed P, Gong Z, Fletcher G, Hew CL. The potential impact of modern biotechnology on fish aquaculture. Aquaculture 2002; 204 (3-4): 255-269. doi: 10.1016/S0044-8486(01)00838-9

7. Dunham RA. Aquaculture and fisheries biotechnology: genetic approaches. CABI Publishing, Cambridge. 2004.

8. Pradeep PJ, Srijaya TC, Bahuleyan A, Papini A. Can sterility through triploidy induction make an impact on Tilapia industry? International Journal of Aquatic Science 2012; 3 (2): 89-96.

9. Pechsiri J, Yakupitiyage A. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 2005; 36 (1): 45-51. doi: 10.1111/j.1365-2109.2004.01182.x

10. Mol K, Byamungu N, Cuisset B, Yaron Z, Ofir M, et al. Hormonal profile of growing male and female diploids and triploids of the blue tilapia (*Oreochromis aureus*) reared in intensive culture. Fish Physiology and Biochemical 1994; 13 (3): 209-218. doi: 10.1007/BF00004359

11. Hussain MG, Rao GPS, Humayun NM, Randall CF, Penman DJ, et al. Comparative performance of growth, biochemical composition and endocrine profiles in diploid and triploid tilapia (*Oreochromis niloticus* L.). Aquaculture 1995; 138 (1-4): 87-97. doi: 10.1016/0044-8486(95)01079-3

12. Puckhaber B, Horstgen-Schwark G. Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). In: Pullin RSV, Lazard M, Legendre JB, Kothlas A, Pauly D (eds): The Third International Symposium on Tilapia in Aquaculture. Manila: ICLARM Conference Proceedings. 1996; pp. 377-382.

13. Bhatta S, Iwai T, Miura T, Huguchi M, Maugars G, et al. Differences between male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). Kathmandu University Journal of Science, Engineering and Technology 2012; 8 (II): 57-65. doi: 10.3126/kuset.v8i2.7326

14. Pradeep PJ, Srijaya TC, Papini A, Chatterji AK. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture 2012; 344-349: 181-187. doi: 10.1016/j.aquaculture.2012.03.006

15. Fuentes-Silva C, Soto-Zarazua GM, Torres-Pacheco I, Flores-Rangel A. Male tilapia production techniques: a mini-review. African Journal of Biotechnology 2013; 12 (36): 5496-5502. doi: 10.5897/AJB11.4119

16. Nguyen CD, David CL. The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in Northern Vietnam. Aquaculture 2000; 184 (3-4): 221-231. doi: 10.1016/S0044-8486(99)00329-4

17. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. Journal of Endocrinology 2001; 170 (1): 27-38. doi: 10.1677/joe.0.1700027

18. Cnaani A, Levavi-Sivan B. Sexual development in fish: practical applications for aquaculture. Sex Development 2009; 3 (2-3): 164-175. doi: 10.1159/000223080

19. Bartley D, Rana K, Immink A. The use of interspecific hybrids in aquaculture and fisheries. Review Fish Biology and Fisheries 2001; 10 (3): 325-337. doi: 10.1023/A:1016691725361

20. Popma TJ, Green BW. Sex reversal of tilapia in earthen ponds. Aquaculture Production Manual, Research and Development Series No. 35, International Center for Aquaculture, Auburn University, Alabama, USA. 1991.

21. Pandian TJ, Sheela SG. Hormonal induction of sex reversal in fish. Aquaculture 1995; 138 (1-4): 1-22. doi: 10.1016/0044-8486(95)01075-0

22. Mukti AT. Optimation of 17α-methyltestosterone synthetic hormone dose and immersion duration in larvae on the success of Nile tilapia (*Oreochromis* sp.) sex reversal. Faculty of Fisheries, Brawijaya University, Malang, Indonesia. 1998.

23. Romerio MP, Fencrich-Verani CSN, Santo De-Copmus BE, Pasilva AS. Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasil Zoology 2000; 29 (3): 654-659. doi: 10.1590/S1516-35982000000300003

24. Mukti AT, Priyambodo B, Rustidja, Widodo MS. Optimization of both 17α-methyltestosterone synthetic hormone dosage and dipping duration of Nile tilapia (*Oreochromis* sp.) larvae on sex reversal efficacy. BIOSAIN Journal of Life Science 2002; 2 (1): 1-8.

25. Mohamed AH, Traifalgar RFM, Serrano Jr. AE, Peralta JP, Pedroso FL. Dietary administration of dehydroepiandrosterone hormone influences the sex differentiation of hybrid red Tilapia (*O. niloticus* × *O. mossambicus*) larvae. Journal of Fisheries and Aquatic Science 2012; 7 (6): 447-453. doi: 10.3923/jfas.2012.44 7.453

26. Beaven U, Muposhi V. Aspects of a monosex population of (*Oreochromis niloticus*) fingerling produced using 17-α methyl testosterone hormone. Journal of Aquaculture Research & Development 2012; 3 (3): 132. doi: 10.4172/2155-9546.1000132

27. Dagne A, Degefu F, Lakew A. Comparative growth performance of monosex and mixed-sex Nile tilapia (*Oreochromis niloticus* L.) in pond culture system at Sebeta, Ethiopian. International Journal of Aquaculture 2013; 3 (7): 30-34. doi: 10.5376/ija.2013.03.0007

28. Ezaz MT, Myers JM, Powell SF, McAndrew BJ, Penman DJ. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 2004; 232 (1-4): 205-214. doi: 10.1016/j.aquaculture.2003.08.001

29. Muller-Belecke A, Horstgen-Schwark G. A YY-male (*Oreochromis niloticus*) strain developed from an exceptional mitotic gynogenetic male and growth performance testing of genetically all-male progenies. Aquaculture Research 2007; 38 (7): 773-775. doi: 10.1111/j.1365-2109.2007.01712.x

30. Aliah RS, Sumantadinata K, Maskur, Naim S. GESIT tilapia: Indonesia's genetic supermales. Global Aquaculture Advocate. May/June. 2010; 36-37.

31. Turra EM, Oliveira DAA, Teixeira EA, Luz RK, Prado SA, et al. Reproduction control in Nile tilapia (*Oreochromis niloticus*) by sexual and chromosome set manipulation. Revista Brasil de Reproduction Animal, Belo Horizonte. 2010; 34 (1): 21-28.

32. Kligerman AD, Bloom SE. Rapid chromosome preparation from solid tissues of fish. Journal of Fisheries Research Board Canada. 1977; 34: 266-269. doi: 10.1139/f77-039

33. Mukti AT, Carman O, Alimuddin, Zairin Jr. M. A rapid chromosome preparation technique without metaphase arrest for ploidy determination in Nile tilapia (*Oreochromis niloticus*). Caryologia 2016; 6 (2): 175-180. doi: 10.1080/00087114.2016.1152112.

34. Buchtova H, Svobodova Z, Kocour M, Velisek J. Evaluation of the dressing percentage of 3-year-old experimental scaly crossbreds of the common carp *Cyprinus carpio* (Linnaeus, 1758) in relation to sex. Acta Veterinary Brno 2006; 75 (1): 123-132. doi: 10.2754/avb200675010123

35. AOAC [Association of Official Analytical Chemists]. Official methods of analysis. (18th eds), Association of Official Analytical Chemists Inc., Washington. 2005.

36. Tave D. Genetics for fish hatchery managers. Avi Publishing, Connecticut. 1993.

37. Mukti AT, Rustidja, Sumitro SB, Djati MS. Polyploidization of common carp (*Cyprinus carpio* L.). BIOSAIN Journal of Life Science 2001; 1 (1): 111-123.

38. Lawson EO, Ishola HA. Effects of cold shock treatment on the survival of fertilized eggs and growth performance of the larvae of African mud catfish *Clarias gariepinus* (Burchell, 1822). Research Journal of Fisheries and Hydrobiology 2010; 5 (2): 85-91.

39. Qin JG, Fast AW, Ako H. Grow-out performance of diploid and triploid Chinese catfish (*Clarias fuscus*). Aquaculture 1998; 166 (3-4): 247-258. doi: 10.1016/S0044-8486(98)00287-7

40. Burke HA, Sacobie CFD, Lall SP, Benfey TJ. The effect of triploidy on juvenile Atlantic salmon (*Salmo salar*) response to varying levels of dietary phosphorus. Aquaculture 2010; 306 (1-4): 295-301. doi: 10.1016/j.aquaculture.2010.05.002

41. Piferrer F, Beaumont A, Falguière J-C, Flajšhans M, Haffray P, et al. Polyploid fish and shellfish: Production, biology, and applications to aquaculture for performance improvement and genetic containment. Aquaculture 2009; 293 (3-4): 125-156. doi: 10.1016/j.aquaculture.2009.04.036

42. Aliah RS, Yamaoka K, Inada Y, Taniguchi N. Effects of triploidy on tissue structure of some organs in ayu. Bulletin Japan Society Science Fisheries 1990; 56 (4): 569-575. doi: 10.2331/suisan.56.569

43. Kerby JH, Eversona JM, Harrell RM, Geiger JG, Starling CC, et al. Performance comparisons between diploid and triploid sunshine bass in freshwater ponds. Aquaculture 2002; 211 (1-4): 91-108. doi: 10.1016/S0044-8486(02)00009-1

44. Cal RM, Vidal S, Gomez C, Ivarez-Blazquez BA, Martinez P, et al. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). Aquaculture 2006; 251 (1): 99-108. doi: 10.1016/j.aquaculture.2005.05.010

45. Felip A, Piferrer F, Zanuy S, Carrillo M. Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons. Journal of Fish Biology 2001; 58 (1): 76-88. doi: 10.1111/j.1095-8649.2001.tb00500.x

46. Taniguchi N, Kijima A, Tamura T, Takegami K, Yamasaki I. Color, growth and maturation in ploidy-manipulated fancy carp. Aquaculture 1986; 57 (1-4): 321-328. doi: 10.1016/0044-8486(86)90210-3

47. Achegbulu CE, Okonji VA, Obi A. Growth and economic performance of diploid and triploid African catfish (*Clarias gariepinus*) in outdoor concrete tanks. International Journal of Genetics 2013; 3 (1): 01-06. doi: 10.5829/idosi.ijg.2013.3.1.738

48. Chen S, Wang J, Liu SJ, Qin QB, Xiao J, et al. Biological characteristics of an improved triploid crucian carp. Science China Series C: Life Science 2009; 52 (8): 733-738. doi: 10.1007/s11427-009-0079-3

49. Tabata YA, Rigolino MG, Tsukamoto RY. Production of all-female triploid rainbow trout (*Oncorhynchus mykiss*) [Pisces, Salmonidae]. III. Growth up to first sexual maturation. Boletim do Instituto de Pesca Sao Paulo 1999; 25: 67-76.

50. Varadaraj K, Pandian TJ. Production of all-female sterile-triploid (*Oreochromis mossambicus*). Aquaculture 1990; 84 (2): 117-123. doi: 10.1016/0044-8486(90)90342-K

51. Felip A, Carrillo M, Zanuy S. Older triploid fish retain impaired reproductive endocrinology in the European sea bass (*Dicentrarchus labrax*). Journal of Fish Biology 2009; 75 (10): 2657-2669. doi: 10.1111/j.1095-8649.2009.02458.x

52. Byamungu N, Darras VM, Kuhn ER. Growth of heat-shock induced triploids of blue tilapia (*Oreochromis aureus*) reared in tanks and in ponds in Eastern Congo: feeding regimes and compensatory growth response of triploid females. Aquaculture 2001; 198 (1-2): 109-122. doi: 10.1016/S0044-8486(00)00605-0

53. Haffray P, Bruant J-S, Facqueur J-M, Fostier A. Gonad development, growth, survival and quality traits in triploids of the protandrous hermaphrodite gilthead sea bream (*Sparus aurata* L.). Aquaculture 2005; 247 (1-4): 107-117. doi: 10.1016/j.aquaculture.2005.02.037

54. Werner C, Poontawee K, Mueller-Belecke A, Horstgen-Schwark G, Wicke M. Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. Archiv Tierzucht 2008; 51 (1): 71-83. doi: 10.5194/aab-51-71-2008

55. Basavaraju Y, Mair GC, Kumar HMM, Kumar SP, Keshavappa GY, et al. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp (*Cyprinus carpio*) in Karnataka, India. Aquaculture 2002; 204 (3-4): 407-418. doi: 10.1016/S0044-8486(01)00827-4

Table 1. The growth, survival rate, and feed conversion ratio performances of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period (n = 20).

Parameter	Fish groups					
	Triploid			Diploid		
	All-male	All-female	Mixed-sex	All-male	All-female	Mixed-sex
Initial biomass (g)	278.6±5.2	190.0±8.3	236.2±6.0	205.0±8.9	136.0±8.8	183.4±5.8
Final biomass (g)	8 056.7±405.5	5 193.3±445.6	7 013.3±551.4	6 130.0±366.6	4 626.7±277.6	5 676.7±465.0
Δ Biomass (g)	7 778.1±404.3^{a}	5 003.0 ±437.9^{e}	6 777.1±548.9^{b}	5 925.0±363.5^{c}	4 490.7±284.9^{f}	5 493.2±462.9^{d}
Δ B 3N - 2N (%)	31.3	11.4	23.4	-	-	-
Initial BW (g)	13.9±0.3	9.5±0.4	11.8±0.3	10.3±0.4	6.8±0.4	9.2±0.3
Final BW (g)	402.8±20.3	278.5±23.2	350.7±27.6	317.0±13.5	252.3±10.2	288.3±15.5
Δ BW (g)	388.9±20.2^{a}	269.0±22.8^{d}	338.9±27.4^{b}	306.7±13.6^{c}	245.5±10.7^{e}	279.2±15.3^{d}
Δ BW 3N - 2 N (%)	26.8	9.6	21.4	-	-	-
Initial BL (mm)	99.2±0.0	93.3±0.0	92.5±0.0	96.3±0.0	92.8±0.0	91.2±0.0
Final BL (mm)	274.5±2.1	241.3±6.7	266.5±5.6	250.0±2.4	232.2±1.9	243.4±4.6
Δ BL (mm)	175.7±2.1^{a}	147.9±6.7^{d}	174.0±5.6^{b}	153.7±2.4^{c}	139.3±1.9^{e}	152.2±4.6^{c}
Δ BL 3N - 2N (%)	14.3	6.2	14.3	-	-	-
AGR (g day^{-1})	3.2±0.2^{a}	2.2±0.2^{d}	2.8±0.2^{b}	2.6±0.1^{c}	2.1±0.1^{e}	2.3±0.1^{d}
Feed conversion ratio	1.2±0.1^{b}	1.4±0.1^{c}	1.1±0.0^{a}	1.2±0.1^{b}	1.4±0.0^{c}	1.4±0.0^{c}
Survival rate (%)	100.0±0.0^{a}	93.3±5.8^{c}	100.0±0.0^{a}	96.7±2.9^{b}	91.7±2.9^{c}	98.3±2.9^{ab}

Note: Δ = gain, Δ B 3N - 2N = relative percentage of triploid:diploid biomass gain, BW = body weight, Δ BW 3N - 2N = relative percentage of triploid:diploid body weight gain, BL = body length, Δ BL 3N - 2N = relative percentage of triploid:diploid body length gain, and AGR = absolute growth rate. Different superscripts in the same row indicates significant differences ($P < 0.05$)

Table 2. Flesh percentages of male and female both triploid and diploid of Nile tilapia fish (n = 10).

Fish group		Body weight (g)	Dressing		Edible carcass	
			Weight (g)	(%)	Weight (g)	(%)
Triploid	♂	414.1±39.2[a]	238.3±19.9[a]	57.6±1.8[b]	170.9±16.0[a]	41.3±1.4[a]
	♀	260.8±24.0[c]	154.0±13.5[c]	59.1±1.6[a]	109.4±10.8[c]	42.0±1.2[a]
Diploid	♂	332.0±29.7[b]	187.2±18.4[b]	56.4±1.6[b]	129.4±12.4[b]	39.0±1.6[b]
	♀	259.4±14.1[c]	141.0±7.8[c]	54.4±1.3[c]	98.5±6.0[d]	38.0±1.4[b]

Note: Different superscripts in the same column indicates significant differences ($P < 0.05$)

Formatted: Font:

Formatted: English (United States)

Table 3. Flesh proximate analysis of male and female both triploid and diploid of Nile tilapia fish (% dry weight) (n = 10).

Fish group		Protein	Lipid	Ash	Carbohydrate
Triploid	♂	85.6 ± 0.3^{ab}	5.1 ± 0.2^{b}	6.2 ± 0.2^{c}	3.2 ± 0.7^{a}
	♀	87.0 ± 1.1^{a}	5.0 ± 0.4^{b}	5.9 ± 0.0^{d}	2.2 ± 1.5^{a}
Diploid	♂	84.2 ± 1.3^{b}	5.9 ± 0.3^{a}	7.1 ± 0.0^{a}	2.8 ± 1.7^{a}
	♀	84.3 ± 1.8^{b}	5.5 ± 0.0^{a}	6.4 ± 0.3^{b}	3.8 ± 1.5^{a}

Note: Different superscripts in the same column indicates significant differences ($P < 0.05$)

Commented [WK10]: There is not enough information on them in M&M.

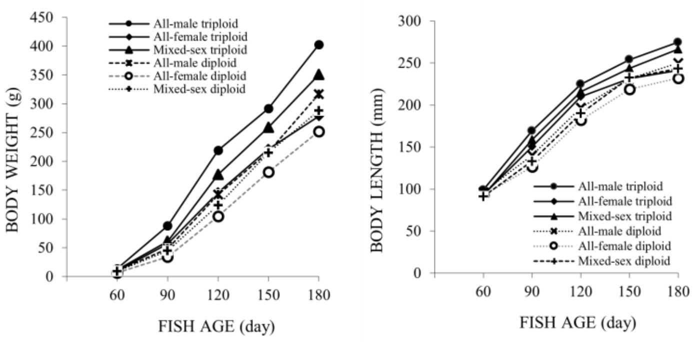

Figure 1. Body weight and body length of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period

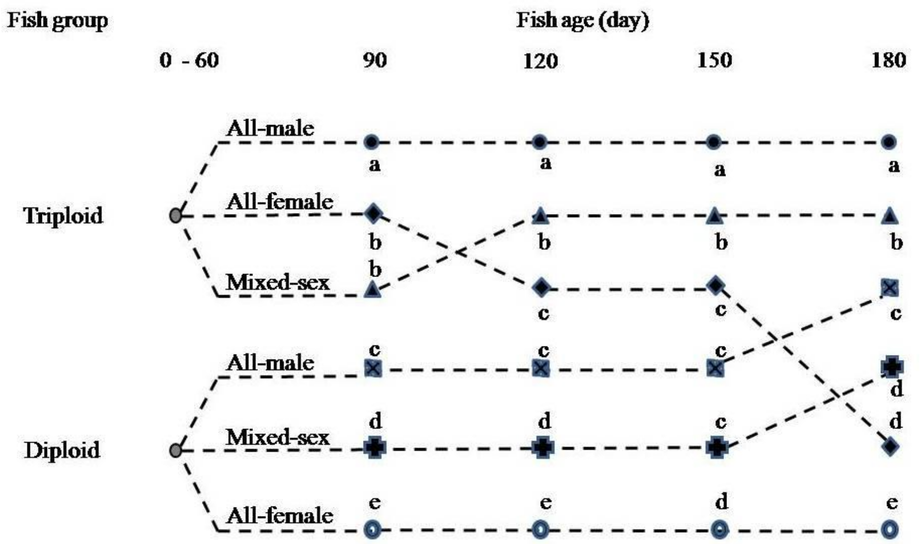

Figure 2. Schematic sequential specific growth rate (SGR) of triploid and diploid Nile tilapia fish during 4 months grow-out period. Different letters at the same fish age indicates significant differences (P < 0.05)

Dear
Editor-in-Chief of Turkish Journal of Veterinary and Aquatic Sciences

Thanks for the corrections and suggestions that have been given to our paper. Authors responses on corrections and suggestions of editor have mentioned in the article with blue-colored words or sentences.

1. The Abstract of article, page 1, line 7: Add "50" before fish.

   Authors response: We have mentioned "50" in the Abstract of article; page 1, line 7: "Before sexing, 50 fish ..."

2. The Materials and methods, page 4, line 6: Add "mg L^{-1}" for dissolved oxygen unit.

   Authors response: We have revised and mentioned "mg L^{-1}"in the Materials and methods of article; page 4, line 21: "...3.4-4.4 mg L^{-1} ..."

3. Editor comment in the Materials and methods, page 5, line 9: Dressing and edible carcass, How was it calculated?

   Authors response: We have revised and mentioned about calculate dressing and edible carcass in the Materials and methods of article; page 6, line 6-13: "The dressing is a piece of fish's body without a head, fins, scales, and internal organs, while the edible carcass is a cut of the right and the left sides of the fish's body. The dressing and edible carcass data were determined according to Buchtova et al. [35] based on ten samples from males and females both triploid and diploid, respectively. The dressing and the edible carcass percentages were calculated by formulas, respectively: ..."

4. Editor comment in the Materials and methods, page 5, line 11: Flesh proximate analysis, Need more explanation.

   Authors response: We have mentioned explanation about flesh proximate contents in the Materials and methods of article; page 7, line 1-3: "In addition, flesh proximate analysis of fish (crude protein, crude lipid, ash, and carbohydrate contents) was evaluated according to AOAC protocol [36] based on ten samples from male and female both triploid and diploid, respectively."

5. Editor comment in the Materials and methods, page 5, line 15: Statistical analysis, Need more detail.

   Authors response: We have revised and mentioned detail sentences in the Materials and methods of article; page 7, line 5-9: "Data on growth performances (biomass gain, body weight and body length gains, AGR, and SGR), FCR, SR, and flesh percentage (dressing and edible carcass percentages), and proximate content were statistically analyzed using the analysis of variance (ANOVA) with SPSS ver.10 software. Duncan's multiple range test was followed by the ANOVA test with a confidence level of 95%."

6. Editor comment in the Materials and methods, page 5, line 17-22 and page 6, line 1: Not right place. These formulas must be given before Statistical analysis in the right place. Please give references.

Authors response: We have revised and mentioned sentences and formulas in the Materials and methods of article; page 5, line 13-22 and page 6, line 1-5: "The growth performances were calculated according to Hariati [34]. The formulas were used to calculate biomass gain (Δ), the relative percentage of triploid : diploid biomass gain, BW gain, the relative percentage of triploid : diploid BW gain, BL gain, the relative percentage of triploid : diploid BL gain, AGR, FCR, SR, and SGR, respectively, as follows: ..."

7. Editor comment in the Results, page 7, line 4: Maturation period, no information in M&M. You have not any measurement.

   Authors response: We have revised and mentioned sentences in the Materials and methods of article; page 5, line 3-5: "In general, the maturation period of tilapia begins after 90-days-old fish. In this study, a maturation period was also observed at the 90th day of fish rearing. The gender of the fish was checked monthly."

8. Editor comment in the Results, page 7, line 23: "8.6 and 10.5% higher than..", Table 2 show different percentage results.

   Authors response: We have mentioned sentences and also formulas to calculate the increase of dressing and edible carcass percentages in the Materials and methods of article; page 6, line 14-18: "Increase of triploid dressing percentage (DP) and edible carcass percentage (ECP) compared to diploid was calculated using the relative percentages of triploid : diploid dressing and edible carcass formulas, respectively, as follows: ..."

   We intend to show the increase dressing and edible carcass percentages of triploid fish than diploid fish, so we have also mentioned sentences about the increase of dressing and edible carcass percentages in the Results of article; page 9, line 4-7: "The increase in dressing and edible carcass percentages of female triploid were 8.6 and 10.5% higher than those of female diploid, respectively. Meanwhile, the increase in dressing and edible carcass percentages of male triploid were 2.1 and 5.9% higher than those of the diploids, respectively (Table 2)."

9. The Discussion of article, page 10, line 5: Add "was" before observed.

   Authors response: We have mentioned "was" in the Discussion of article; page 11, line 9: "The lowest growth was observed ..."

10. Editor comment in the Discussion, page 11, line 1-5: "Triploid Nile tilapia tends to be high in crude protein and low in crude lipid and ash compared to diploid. In terms of sex, male and female fish from both triploid and diploid show the same crude protein, crude lipid and carbohydrates contents, while the ash content was significantly different. This result showed that triploidy in Nile tilapia affects flesh quality, especially crude lipid and ash contents. Further study is needed to gather more valuable information." Discussion with other author's result.

    Authors response: We have mentioned similar result by other authors or researchers in the Discussion of article; page 12, line 9-10: "Triploid Nile tilapia tends to be high in crude protein and low in crude lipid and ash compared to diploid. In terms of sex, male and female fish from both triploid and diploid show the same crude protein, crude lipid and carbohydrates contents, while the ash content was significantly different. This result showed that triploidy in Nile tilapia affects flesh quality, especially crude lipid and ash contents. This result indicated that as well as a study conducted by other researchers [5,6,11]. Further study is needed to gather more valuable information"

11. Editor comment in the Table 3, page 22: Contents of protein, lipid, ash, and carbohydrate, There is not enough information on them in M&M.

    Authors response: We have revised and mentioned the proximate contents in the Materials and methods of article; pages 7, line 1-2: "In addition, flesh proximate analysis of fish (crude protein, crude lipid, ash, and carbohydrate contents) was evaluated according to AOAC protocol [36] ..."

12. Authors have revised all comments and suggestions from Editor about references (new authors response for new editor comments)

Thus authors responses on comments, corrections, and suggestions of editor, we expect the editor were pleased and understand it and we hope that this article will be corrected further. Thank you very much.

Best regards,

Akhmad Taufiq MUKTI

Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)

Abstract: This study aimed to compare the growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia. The triploid population was obtained through heat shock at 41 ºC for 4 minutes, 4 minutes after fertilization. Before sexing, 50 fish were reared in aquaria at a density of 1 fish L^{-1} for 2 months. After sexing, both triploid and diploid fish were grouped into all-male, all-female, and mixed-sex groups and reared in hapas at a density of 10 fish m^{-2} for 4 months. Each group was replicated three times. The highest body weight, body length, and growth rate were observed in all-male triploid, while the lowest of those parameters were obtained in all-female diploid. The highest survival rate was achieved in both all-male and mixed-sex triploids and did not significantly differ from the mixed-sex diploid ($P > 0.05$). The triploid fish had higher edible carcass percentage than diploid. The proximate analysis indicated that the crude protein content of triploid was higher than that of diploid, while the crude lipid and ash contents were lower than those of diploid ($P < 0.05$). Triploid Nile tilapia had the best growth performances, including flesh quantity and quality compared to diploid.

Keywords: Growth performance, triploid production, monosex, mixed-sex, Nile tilapia

1. Introduction

Sterile fish is beneficial in aquaculture because, in the sterile metabolism processes, the fish will reduce or even prevent the use of energy for reproduction. As a result, most of the anabolic energy will be transferred to somatic growth. Sterile fish also have the potential for a better survival rate compared to diploid fish. Devlin et al. [1] stated that the increase in the growth of fish brings substantial benefits in shortening culture period, improving the efficiency of feed utilization and the efficiency of production, and ensuring product availability. Also, culturing sterile fish is one of the best farming management in aquaculture practices, as it enables the use of the metabolism pathway to reach fast somatic tissue instead of producing either sperm or eggs in the spawning season [2].

The high ability (uncontrolled) of tilapia reproduction cause the unexpected density in the pond with varied size and slow growth, making it less commercially profitable in aquaculture. The sterilization is the best possible solution to solve the problems in the tilapia culture [3]. Lutz [4] mentioned that among future's aquaculture commodities, tilapia is a candidate fish to produce functionally sterile seeds on a large scale. The induction of triploidy is one of the methods of producing sterile fish. The culture of triploid fish could provide benefits, such as increased growth, carcass production, survival rate, and flesh quality [5-7].

The production of triploid tilapia has been developed for more than four decades, and triploidy is an effective management tool in tilapia farming in the future [8]. Triploid tilapia has small testis or ovaries, low gonad weight and high body weight, protein utilization, and protein efficiency ratio compared to diploid tilapia. Thus, farming is possibly beneficial [9]. In some cases, the growth performances of triploid tilapia were reported to be superior or equal to those of diploid tilapia [10-12].

On the other hand, some studies indicated that male tilapia has faster growth compared to female tilapia [13-15]. The production level of monosex male tilapia farming was 10% higher compared to the mixed-sex population [16,17]. Associated with presence of sexual dimorphism in terms of growth, many efforts were made to produce all-male seed population for the purpose of monosex culture, which generally can be obtained through four common methods, namely manual sexing [18] at body size of 5-7 cm, hybridization [7,19], hormonal treatments [15,20-27] or chromosome set manipulations, such as androgenesis [18,28] to produce YY supermale parent stocks [29-31].

So far, the combined effects of triploidy and growth-related sexual dimorphism superiorities in tilapia are still unknown. A strain of fish, including tilapia, also possibly influence growth performance during the culture period. Therefore, the present study tries to clarify the effect of those superiorities on growth, survival rate, flesh percentage, and proximate composition of Nile tilapia during the grow-out period.

2. Materials and methods

2.1. Experimental fish preparation

In this study, fish used was the Wanayasa strain of Nile tilapia known as NIRWANA produced through family selection program between the genetic improvement for farmed tilapia (GIFT) and the genetically enhanced tilapia (GET) in Indonesia. The broodstocks were obtained from the Tilapia and Common Carp Aquaculture Development Agency in Purwakarta, West Java, Indonesia. Artificially fertilized eggs (4 minutes after insemination) were subjected to heat shock treatment at 41 ºC for 4 minutes to produce triploid fish. This treatment was produced triploid Nile tilapia of 91-100%, as identified using the chromosome counting method prepared according to Kligerman and Bloom [32] and Mukti et al. [33]. Embryos were incubated in glass funnel in a recirculating system and diploid fish were produced using a similar procedure.

Larvae of both triploid and diploid were separately reared in 50-L aquaria at a density of 1 fish L^{-1}. A total of 10 aquaria were used for triploid and diploid fish, respectively. The 2-days-old fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Next, fish were transferred into 180-L aquaria, reared at a density of 4 fish L^{-1} and fed on a commercial diet (40% crude protein content) for 30 days. Sexing was conducted morphologically by observing the genital openings on the average fish weight of 6.5-10 g to separate males and females of both triploid and diploid fish. The sexing was also confirmed by gonad preparation and observation using the squash method with acetocarmine stain. Twenty fish from different groups, namely all-male triploid, all-female triploid, mixed-sex triploid, all-male diploid, all-female diploid, and mixed-sex diploid were respectively prepared for performance evaluation.

2.2. Performances evaluation

Previously prepared all-male, all-female, and mixed-sex of both triploid and diploid were separately transferred and reared in 2.0 m × 1.0 m × 0.7 m dimensions of floating net (mesh size of 10 mm) placed in a 20 m × 10 m × 1.5 m dimensions of concrete pond at a density of 10 fish m^{-2} with the water exchange rate of 1 L s^{-1}. On the other hand, water qualities, such as temperature, dissolved oxygen, and pH were measured every week with ranges of 27-29 ºC, 3.4-4.4 mg L^{-1}, and 6.7-7.3, respectively. Three floating nets were used as replication for each group. Firstly, fish were fed on a 1-mm-diameter commercial diet (40% crude protein content) at satiation for 30 days, then they were fed on a 3-mm-diameter commercial diet (33% crude protein content) at satiation during the last 3 months (90 days), three times a day.

In general, the maturation period of tilapia begins after 90-days-old fish. In this study, a maturation period was also observed at the 90th day of fish rearing. The gender of the fish was checked monthly. Body weight (BW), body length (BL), mortality, and feed intake data were measured every month. Biomass gain, the relative percentages of biomass, BW, and BL gains triploid compared to diploid, BW and BL gains, absolute growth rate (AGR), feed conversion ratio (FCR), and survival rate (SR) were analyzed based on data of initial and final grow-outs, except specific growth rate (SGR) was analyzed every month during 4 months grow-out of fish, while dressing, edible carcass, and proximate data of male and female both triploid and diploid fish were analyzed at the end of the experimental period.

The growth performances were calculated according to Hariati [34]. The formulas were used to calculate biomass gain (Δ), the relative percentage of triploid:diploid biomass gain, BW gain, the relative percentage of triploid:diploid BW gain, BL gain, the relative percentage of triploid:diploid BL gain, AGR, FCR, SR, and SGR, respectively, as follows:

$$\Delta \text{ Biomass (g)} = \text{Final biomass (g) - initial biomass (g)}$$

$$\Delta \text{ B 3N:2N (\%)} = \frac{\Delta \text{ biomass of triploid (g) - } \Delta \text{ biomass of diploid (g)}}{\Delta \text{ biomass of diploid (g)}} \times 100$$

$$\Delta \text{ BW (g)} = \text{Final body weight (g) - initial body weight (g)}$$

$$\Delta \text{ BW 3N:2N (\%)} = \frac{\Delta \text{ BW of triploid (g) - } \Delta \text{ BW of diploid (g)}}{\Delta \text{ BW of diploid (g)}} \times 100$$

$$\Delta \text{ BL (mm)} = \text{Final body length (mm) - initial body length (mm)}$$

$$\Delta \text{ BL 3N:2N (\%)} = \frac{\Delta \text{ BL of triploid (mm) - } \Delta \text{ BL of diploid (mm)}}{\Delta \text{ BL of diploid (mm)}} \times 100$$

$$\text{AGR (g day}^{-1}) = \frac{\text{Final body weight (g) - initial body weight (g)}}{\text{Long time of rearing (day)}}$$

$$\text{FCR} = \frac{\text{Feed consumed by fish (g)}}{\Delta \text{ body weight of fish (g)}}$$

$$\text{SR (\%)} = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

$$\text{SGR (\% day}^{-1}) = \frac{\text{Ln final body weight - Ln initial body weight}}{\text{Long time of rearing (day)}} \times 100$$

The dressing is a piece of fish's body without a head, fins, scales, and internal organs, while the edible carcass is a cut of the right and the left sides of the fish's body. The dressing and edible carcass data were determined according to Buchtova et al. [35] based on ten samples from males and females both triploid and diploid, respectively. The dressing and the edible carcass percentages were calculated by formulas, respectively:

$$\text{Dressing (\%)} = \frac{\text{Dressing weight of fish}}{\text{Body weight of fish}} \times 100$$

$$\text{Edible carcass (\%)} = \frac{\text{Edible carcass weight of fish}}{\text{Body weight of fish}} \times 100$$

Increase of triploid dressing percentage (DP) and edible carcass percentage (ECP) compared to diploid was calculated using the relative percentages of triploid:diploid dressing and edible carcass formulas, respectively, as follows:

$$\Delta \text{ Dressing 3N:2N (\%)} = \frac{\text{DP of triploid (\%) - DP of diploid (\%)}}{\text{DP of diploid (\%)}} \times 100$$

$$\Delta \text{ Edible carcass 3N:2N (\%)} = \frac{\text{ECP of triploid (\%) - ECP of diploid (\%)}}{\text{ECP of diploid (\%)}} \times 100$$

In addition, flesh proximate analysis of fish (crude protein, crude lipid, ash, and carbohydrate contents) was evaluated according to AOAC protocol [36] based on ten samples from male and female both triploid and diploid, respectively.

2.3. Statistical analysis

Data on growth performances (biomass gain, body weight and body length gains, AGR, and SGR), FCR, SR, and flesh percentage (dressing and edible carcass percentages), and proximate content were statistically analyzed using the analysis of variance (ANOVA) with SPSS ver.10 software. Duncan's multiple range test was followed by the ANOVA test with a confidence level of 95%.

3. Results

3.1. Growth performance, survival rate, and feed conversion ratio

The growth performances of the tested fish groups are shown in Table 1. The results showed that the growth of triploid fish was significantly higher ($P < 0.05$) compared to that of diploid. The biomass gains (Δ B 3N:2N) of all-male, all-female, and mixed-sex triploids fish were 31.3, 11.4, and 23.4% higher than those of diploid, respectively. A similar pattern was found in body weight gain (Δ BW 3N:2N) and body length gain (Δ BL 3N:2N). The highest values of body weight and length gains (26.8 and 14.3%, respectively) were observed in all-male triploid, followed by mixed-sex triploid (21.4 and 14.3%, respectively), while the lowest values (9.6 and 6.2%, respectively) were seen in all-female triploid. Furthermore, all-female diploid fish significantly showed the most inferior growth performance compared to other groups.

All-male triploid had the highest absolute growth rate (AGR) than other groups, followed by mixed-sex triploid, then all-male and all-female diploids. Meanwhile, the mixed-sex triploid had the best feed conversion ratio, followed by all-male triploid and diploid. The survival rates of all-male and mixed-sex triploids and mixed-sex diploid were higher compared to other groups, as shown in Table 1.

Figure 1 shows the monthly body weight and body length recorded during the 4 months grow-out period. In general, triploid grew faster than diploid, and all-male triploid showed the highest growth rate, while all-female diploid showed the lowest growth rate.

In this study, it was observed that in both triploid and diploid fish, males grew faster than females during the experiment. In triploid and diploid groups, the biomass gains of the male were 55.5 and 31.9% higher than those of females, respectively. Before the maturation period, the average body weight of triploid and diploid males was 16.6 and 10.7 g bigger than those of triploid and diploid females, respectively. Meanwhile, during the maturation period, the average body weight of triploid and diploid males was 103.3 and 50.5 g bigger than those of triploid and diploid females, respectively. These results showed that the role of the sexual dimorphism on growth in Nile tilapia had a similar pattern with the role of the ploidy level, the effects of which were highly significant during the maturation period.

All-female and mixed-sex triploids groups showed a similar growth rate at the 90th day (Figure 2). The mixed-sex triploid group has higher specific growth rate (SGR) than other sex groups at the 120th to 180th day, while the all-female triploid group has similar SGR as an all-male diploid group at the 120th day. On the other hand, all-female triploid and all-male and mixed-sex diploids groups have similar SGR at the 150th day. Meanwhile, the all-female triploid group has a similar SGR as the mixed-sex diploid group at the 180th day (Figure 2).

3.2. Flesh percentage and proximate composition

The edible carcass percentages of male and female triploids were higher than those of diploids. The highest and lowest dressing percentages were found in triploid and diploid females, respectively ($P < 0.05$). The increase in dressing and edible carcass percentages of female triploid were 8.6 and 10.5% higher than those of female diploid, respectively. Meanwhile, the increase in dressing and edible carcass percentages of male triploid were 2.1 and 5.9% higher than those of the diploids, respectively (Table 2).

Flesh proximate analysis of triploid and diploid fish is shown in Table 3. The crude protein content of female triploid was similar to that of male triploid, however, it was higher than that of diploid fish ($P < 0.05$). On the other hand, crude lipid and ash contents of male and female triploids were lower than diploids. There were no significant differences in carbohydrate content between triploid and diploid fish.

4. Discussion

This study revealed that ploidy level and sexual dimorphism play essential roles in Nile tilapia growth performance. The high growth of male triploid and low growth of female diploid indicated that both ploidy level and sexual dimorphism significantly affected Nile tilapia growth (Table 1 and Figures 1 and 2).

Tave [37] reported that triploidization leads to an increase in sterility and growth. A cell size of triploid is larger than diploid, and energy for gamete production is reduced or inhibited. In most cases, triploid showed heavier body size and faster growth than diploid in common carp (*Cyprinus carpio*) [38], African mud catfish (*Clarias gariepinus*) [39], Chinese catfish (*C. fuscus*) [40], and Atlantic salmon (*Salmo salar*) [41]. Besides, the performances of triploid fish were not only species and age-dependent but also depended on the experimental conditions and the interactions between the environment and genetics [7]. The individual body size of triploid was more significant due to the larger cell size compared to diploid [42]. However, Aliah et al. [43] reported that the cell size was not correlated with the organ size in sticklebacks (*Gasterosteus aculeatus*). Furthermore, in 2-3 month-old sunshine bass (*Morone* spp.), diploid grew faster compared to triploid [44].

The increase in triploid growth is due to the influence of sterility, diverting energy (nutrient) for somatic growth rather than gonadal development and sexual activity [14]. Most studies concluded that the significant difference in growth rate between triploid and diploid fish occurred during the maturation period in fish such as turbot (*Scophthalmus maximus*) [45] and European sea bass (*Dicentrarchus labrax*) [46]. In this study, it was found that the growth difference (30.0%) between triploid and diploid fish already occurred before (≤ 90-days-old) and during the maturation period (90- to 180-day-old). Also, the growth of triploid showed more significant differences compared to diploid (39.3%). A similar phenomenon has been reported in fancy carp (*C. carpio*) [47].

The role of sexual dimorphism in growth in tilapia has been revealed in the last three decades. Male tilapia grew faster compared to females, so the all-male monosex culture in this species is worldwide applied. Similar cases were found in catfish (*C. gariepinus*) [48] and crucian carp (*Carassius auratus*) [49].

The comparison of the growth performance among the six groups showed that all-male triploid and all-female diploid fish grew faster and lower, respectively than the fish in other groups during the experiment. The interaction effect between triploidy and sexual dimorphism in growth was not significant among all-female triploid, all-male diploid, and mixed-sex diploid groups at the 120th to 150th day. In the same groups, all-male diploid grew faster than the others and the interaction effect between triploidy and sexual dimorphism on growth was not significant among all-female triploid and mixed-sex diploid at the 180th day (Figure 2). This phenomenon seemed to be species-specific as found in rainbow trout (*Oncorhynchus mykiss*) by Tabata et al. [50], Mozambique tilapia (*O. mossambicus*) by Varadaraj and Pandian [51] and European sea bass by Felip et al. [52]. Those authors reported that female triploid grew faster than either male triploid, male and female diploids or mixed-sex diploid.

The lowest growth was observed in all-female diploid looked as if the female diploid went through rapid reproductive development and sexual maturity. So, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth. In this study, it was recorded that at the 120th day, the majority of female diploid began to spawn and incubate either fertilized or unfertilized eggs in the mouth. This aspect generally allows the female to not feed during eggs incubation for 15 days until larvae can swim freely, as reported by Byamungu et al. [53]. In other words, the role of the ploidy level in growth during the maturation period was significantly higher than that before the maturation period. These results also revealed that a high body weight gain in male and female triploid during maturation period seemed to be due to the sterility of triploid fish and reproductive activity of diploid fish.

In this study, triploid fish had higher flesh percentages compared to diploid, and female triploid also had higher flesh percentages. Similar results were reported in gilthead sea bream (*Sparus aurata*) [54] and rainbow trout [55]. However, in common carp [56] up to the size of 400 g, the dressing weight of triploid was not significantly different from that of diploid. The results of this study indicated that higher flesh percentages of female triploid compared to male triploid was because the female was more sterile than male, while the higher flesh percentages in triploid compared to diploid seemed correlated with normal in diploid and reducing in triploid through gonadal developments.

Triploid Nile tilapia tends to be high in crude protein and low in crude lipid and ash compared to diploid. In terms of sex, male and female fish from both triploid and diploid show the same crude protein, crude lipid and carbohydrates contents, while the ash content was significantly different. This result showed that triploidy in Nile tilapia affects flesh quality, especially crude lipid and ash contents. This result indicated that as well as a study conducted by other researchers [5,6,11]. Further study is needed to gather more valuable information.

The interaction effect between triploidy and sexual dimorphism strongly related to growth had a positive contribution to production performance, especially during the maturation period. Based on the examination of various aspects related to production, the result revealed that all-male triploid Nile tilapia culture has the potential to be developed. Hence, in the future, an applicable method for mass all-male triploid seed production should be considered. One of the possible strategic efforts is how to produce supermale tetraploid as parent stock by combining the chromosome set and hormonal manipulations.

References

1. Devlin R, Biagi CA, Yesaki TY. Growth, viability and genetic characteristics of GH transgenic Coho salmon strains. Aquaculture 2004; 236 (1-4): 607-632. doi:10.1016/j.aquaculture.2004.02.026

2. Galli L. Genetic Modification in Aquaculture: A Review of Potential Benefits and Risks. Bureau of Rural Sciences, Australia: Canberra; 2002.

3. Pradeep PJ, Srijaya TC, Jose D, Papini A, Hassan A, et al. Identification of diploid and triploid red tilapia by using erythrocyte indices. Caryologia 2011; 64 (4): 485-492. doi:10.1080/00087114.2011.10589816

4. Lutz CG. Practical Genetics for Aquaculture. Fishing News Books, Oxford: Blackwell Science; 2001.

5. Felip A, Zanuy S, Carrillo M, Piferrer F. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 2001; 111 (1-3): 175-195.

6. Melamed P, Gong Z, Fletcher G, Hew CL. The potential impact of modern biotechnology on fish aquaculture. Aquaculture 2002; 204 (3-4): 255-269. doi:10.1016/S0044-8486(01)00838-9

7. Dunham RA. Aquaculture and Fisheries Biotechnology: Genetic Approaches. Cambridge: CABI Publishing; 2004.

8. Pradeep PJ, Srijaya TC, Bahuleyan A, Papini A. Can sterility through triploidy induction make an impact on Tilapia industry? International Journal of Aquatic Science 2012; 3 (2): 89-96.

9. Pechsiri J, Yakupitiyage A. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 2005; 36 (1): 45-51. doi:10.1111/j.1365-2109.2004.01182.x

10. Mol K, Byamungu N, Cuisset B, Yaron Z, Ofir M, et al. Hormonal profile of growing male and female diploids and triploids of the blue tilapia (*Oreochromis*

aureus) reared in intensive culture. Fish Physiology and Biochemical 1994; 13 (3): 209-218. doi:10.1007/BF00004359

11. Hussain MG, Rao GPS, Humayun NM, Randall CF, Penman DJ, et al. Comparative performance of growth, biochemical composition, and endocrine profiles in diploid and triploid tilapia (*Oreochromis niloticus* L.). Aquaculture 1995; 138 (1-4): 87-97. doi:10.1016/0044-8486(95)01079-3
12. Puckhaber B, Horstgen-Schwark G. Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). In: ICLARM Conference Proceedings of The Third International Symposium on Tilapia in Aquaculture. Manila; 1996. pp. 377-382.
13. Bhatta S, Iwai T, Miura T, Huguchi M, Maugars G, et al. Differences between male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). Kathmandu University Journal of Science, Engineering, and Technology 2012; 8 (II): 57-65. doi:10.3126/kuset.v8i2.7326
14. Pradeep PJ, Srijaya TC, Papini A, Chatterji AK. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture 2012; 344-349: 181-187. doi:10.1016/j.aquaculture.2012.03.006
15. Fuentes-Silva C, Soto-Zarazua GM, Torres-Pacheco I, Flores-Rangel A. Male tilapia production techniques: a mini-review. African Journal of Biotechnology 2013; 12 (36): 5496-5502. doi:10.5897/AJB11.4119
16. Nguyen CD, David CL. The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis*

niloticus) in Northern Vietnam. Aquaculture 2000; 184 (3-4): 221-231. doi:10.1016/S0044-8486(99)00329-4

17. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. Journal of Endocrinology 2001; 170 (1): 27-38. doi:10.1677/joe.0.1700027
18. Cnaani A, Levavi-Sivan B. Sexual development in fish: practical applications for aquaculture. Sex Development 2009; 3 (2-3): 164-175. doi:10.1159/000223080
19. Bartley D, Rana K, Immink A. The use of interspecific hybrids in aquaculture and fisheries. Review Fish Biology and Fisheries 2001; 10 (3): 325-337. doi:10.1023/A:1016691725361
20. Popma TJ, Green BW. Sex Reversal of Tilapia in Earthen Ponds. Aquaculture Production Manual, Research and Development Series No. 35, International Center for Aquaculture, Auburn University, USA: Alabama; 1991.
21. Pandian TJ, Sheela SG. Hormonal induction of sex reversal in fish. Aquaculture 1995; 138 (1-4): 1-22. doi:10.1016/0044-8486(95)01075-0
22. Mukti AT. Optimation of 17α-methyltestosterone synthetic hormone dose and immersion duration in larvae on the success of Nile tilapia (*Oreochromis* sp.) sex reversal. BSc, Faculty of Fisheries, Brawijaya University, Malang, Indonesia, 1998 (in Indonesian).
23. Romerio MP, Fencrich-Verani CSN, Santo De-Copmus BE, Pasilva AS. Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasil Zoology 2000; 29 (3): 654-659. doi:10.1590/S1516-35982000000300003

24. Mukti AT, Priyambodo B, Rustidja, Widodo MS. Optimization of both 17α-methyltestosterone synthetic hormone dosage and dipping duration of Nile tilapia (*Oreochromis* sp.) larvae on sex reversal efficacy. BIOSAIN Journal of Life Science 2002; 2 (1): 1-8 (in Indonesian with an abstract in English).

25. Mohamed AH, Traifalgar RFM, Serrano Jr. AE, Peralta JP, Pedroso FL. Dietary administration of dehydroepiandrosterone hormone influences the sex differentiation of hybrid red Tilapia (*O. niloticus* × *O. mossambicus*) larvae. Journal of Fisheries and Aquatic Science 2012; 7 (6): 447-453. doi:10.3923/jfas.2012.447.453

26. Beaven U, Muposhi V. Aspects of a monosex population of (*Oreochromis niloticus*) fingerling produced using 17-α methyltestosterone hormone. Journal of Aquaculture Research and Development 2012; 3 (3): 132. doi:10.4172/2155-9546.1000132

27. Dagne A, Degefu F, Lakew A. Comparative growth performance of monosex and mixed-sex Nile tilapia (*Oreochromis niloticus* L.) in pond culture system at Sebeta, Ethiopian. International Journal of Aquaculture 2013; 3 (7): 30-34. doi:10.5376/ija.2013.03.0007

28. Ezaz MT, Myers JM, Powell SF, McAndrew BJ, Penman DJ. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 2004; 232 (1-4): 205-214. doi:10.1016/j.aquaculture.2003.08.001

29. Muller-Belecke A, Horstgen-Schwark G. A YY-male (*Oreochromis niloticus*) strain developed from an exceptional mitotic gynogenetic male and growth

performance testing of genetically all-male progenies. Aquaculture Research 2007; 38 (7): 773-775. doi:10.1111/j.1365-2109.2007.01712.x

30. Aliah RS, Sumantadinata K, Maskur, Naim S. GESIT tilapia: Indonesia's genetic supermales. Global Aquaculture Advocate, May/June 2010; 36-37.

31. Turra EM, Oliveira DAA, Teixeira EA, Luz RK, Prado SA, et al. Reproduction control in Nile tilapia (*Oreochromis niloticus*) by sexual and chromosome set manipulation. Revista Brasil de Reproduction Animal, Belo Horizonte 2010; 34 (1): 21-28.

32. Kligerman AD, Bloom SE. Rapid chromosome preparation from solid tissues of fish. Journal of Fisheries Research Board Canada 1977; 34: 266-269. doi:10.1139/f77-039

33. Mukti AT, Carman O, Alimuddin, Zairin Jr. M. A rapid chromosome preparation technique without metaphase arrest for ploidy determination in Nile tilapia (*Oreochromis niloticus*). Caryologia 2016; 6 (2): 175-180. doi:10.1080/00087114.2016.1152112

34. Hariati AM. Fish Feed. Nuffic/Unibraw/Luw/Fish Fisheries Project, Malang: Brawijaya University; 1989 (in Indonesian).

35. Buchtova H, Svobodova Z, Kocour M, Velisek J. Evaluation of the dressing percentage of 3-year-old experimental scaly crossbreds of the common carp *Cyprinus carpio* (Linnaeus, 1758) in relation to sex. Acta Veterinary Brno 2006; 75 (1): 123-132. doi:10.2754/avb200675010123

36. AOAC [Association of Official Analytical Chemists]. Official Methods of Analysis. 18th ed. Washington: Association of Official Analytical Chemists Inc.; 2005.

37. Tave D. Genetics for Fish Hatchery Managers. Connecticut: Avi Publishing; 1993.

38. Mukti AT, Rustidja, Sumitro SB, Djati MS. Polyploidization of common carp (*Cyprinus carpio* L.). BIOSAIN Journal of Life Science 2001; 1 (1): 111-123 (in Indonesian with an abstract in English).

39. Lawson EO, Ishola HA. Effects of cold shock treatment on the survival of fertilized eggs and growth performance of the larvae of African mud catfish *Clarias gariepinus* (Burchell, 1822). Research Journal of Fisheries and Hydrobiology 2010; 5 (2): 85-91.

40. Qin JG, Fast AW, Ako H. Grow-out performance of diploid and triploid Chinese catfish (*Clarias fuscus*). Aquaculture 1998; 166 (3-4): 247-258. doi:10.1016/S0044-8486(98)00287-7

41. Burke HA, Sacobie CFD, Lall SP, Benfey TJ. The effect of triploidy on juvenile Atlantic salmon (*Salmo salar*) response to varying levels of dietary phosphorus. Aquaculture 2010; 306 (1-4): 295-301. doi:10.1016/j.aquaculture.2010.05.002

42. Piferrer F, Beaumont A, Falguière J-C, Flajšhans M, Haffray P, et al. Polyploid fish and shellfish: production, biology, and applications to aquaculture for performance improvement and genetic containment. Aquaculture 2009; 293 (3-4): 125-156. doi:10.1016/j.aquaculture.2009.04.036

43. Aliah RS, Yamaoka K, Inada Y, Taniguchi N. Effects of triploidy on tissue structure of some organs in ayu. Bulletin Japan Society Science Fisheries 1990; 56 (4): 569-575. doi:10.2331/suisan.56.569

44. Kerby JH, Eversona JM, Harrell RM, Geiger JG, Starling CC, et al. Performance comparisons between diploid and triploid sunshine bass in freshwater ponds. Aquaculture 2002; 211 (1-4): 91-108. doi:10.1016/S0044-8486(02)00009-1

45. Cal RM, Vidal S, Gomez C, Ivarez-Blazquez BA, Martinez P, et al. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). Aquaculture 2006; 251 (1): 99-108. doi:10.1016/j.aquaculture.2005.05.010

46. Felip A, Piferrer F, Zanuy S, Carrillo M. Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons. Journal of Fish Biology 2001; 58 (1): 76-88. doi:10.1111/j.1095-8649.2001.tb00500.x

47. Taniguchi N, Kijima A, Tamura T, Takegami K, Yamasaki I. Color, growth, and maturation in ploidy-manipulated fancy carp. Aquaculture 1986; 57 (1-4): 321-328. doi:10.1016/0044-8486(86)90210-3

48. Achegbulu CE, Okonji VA, Obi A. Growth and economic performance of diploid and triploid African catfish (*Clarias gariepinus*) in outdoor concrete tanks. International Journal of Genetics 2013; 3 (1): 01-06. doi:10.5829/idosi.ijg.2013.3.1.738

49. Chen S, Wang J, Liu SJ, Qin QB, Xiao J, et al. Biological characteristics of an improved triploid crucian carp. Science China Series C: Life Science 2009; 52 (8): 733-738. doi:10.1007/s11427-009-0079-3

50. Tabata YA, Rigolino MG, Tsukamoto RY. Production of all-female triploid rainbow trout (*Oncorhynchus mykiss*) [Pisces, Salmonidae]. III. Growth up to first sexual maturation. Boletim do Instituto de Pesca Sao Paulo 1999; 25: 67-76.

51. Varadaraj K, Pandian TJ. Production of all-female sterile-triploid (*Oreochromis mossambicus*). Aquaculture 1990; 84 (2): 117-123. doi:10.1016/0044-8486(90)90342-K

52. Felip A, Carrillo M, Zanuy S. Older triploid fish retain impaired reproductive endocrinology in the European sea bass (*Dicentrarchus labrax*). Journal of Fish Biology 2009; 75 (10): 2657-2669. doi:10.1111/j.1095-8649.2009.02458.x

53. Byamungu N, Darras VM, Kuhn ER. Growth of heat-shock induced triploids of blue tilapia (*Oreochromis aureus*) reared in tanks and in ponds in Eastern Congo: feeding regimes and compensatory growth response of triploid females. Aquaculture 2001; 198 (1-2): 109-122. doi:10.1016/S0044-8486(00)00605-0

54. Haffray P, Bruant J-S, Facqueur J-M, Fostier A. Gonad development, growth, survival and quality traits in triploids of the protandrous hermaphrodite gilthead sea bream (*Sparus aurata* L.). Aquaculture 2005; 247 (1-4): 107-117. doi:10.1016/j.aquaculture.2005.02.037

55. Werner C, Poontawee K, Mueller-Belecke A, Horstgen-Schwark G, Wicke M. Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. Archiv Tierzucht 2008; 51 (1): 71-83. doi:10.5194/aab-51-71-2008

56. Basavaraju Y, Mair GC, Kumar HMM, Kumar SP, Keshavappa GY, et al. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp (*Cyprinus carpio*) in Karnataka, India. Aquaculture 2002; 204 (3-4): 407-418. doi:10.1016/S0044-8486(01)00827-4

Table 1. The growth, survival rate, and feed conversion ratio performances of sex-grouped triploid and diploid Nile tilapia fish during 4 months grow-out period (n = 20).

Parameter	Fish groups					
	Triploid			Diploid		
	All-male	All-female	Mixed-sex	All-male	All-female	Mixed-sex
Initial biomass (g)	278.6±5.2	190.0±8.3	236.2±6.0	205.0±8.9	136.0±8.8	183.4±5.8
Final biomass (g)	8056.7±405.5	5193.3±445.6	7013.3±551.4	6130.0±366.6	4626.7±277.6	5676.7±465.0
Δ Biomass (g)	7778.1±404.3^{a}	5003.0 ±437.9^{e}	6777.1±548.9^{b}	5925.0±363.5^{c}	4490.7±284.9^{f}	5493.2±462.9^{d}
Δ B 3N:2N (%)	31.3	11.4	23.4	-	-	-
Initial BW (g)	13.9±0.3	9.5±0.4	11.8±0.3	10.3±0.4	6.8±0.4	9.2±0.3
Final BW (g)	402.8±20.3	278.5±23.2	350.7±27.6	317.0±13.5	252.3±10.2	288.3±15.5
Δ BW (g)	388.9±20.2^{a}	269.0±22.8^{d}	338.9±27.4^{b}	306.7±13.6^{c}	245.5±10.7^{e}	279.2±15.3^{d}
Δ BW 3N:2N (%)	26.8	9.6	21.4	-	-	-
Initial BL (mm)	99.2±0.0	93.3±0.0	92.5±0.0	96.3±0.0	92.8±0.0	91.2±0.0
Final BL (mm)	274.5±2.1	241.3±6.7	266.5±5.6	250.0±2.4	232.2±1.9	243.4±4.6
Δ BL (mm)	175.7±2.1^{a}	147.9±6.7^{d}	174.0±5.6^{b}	153.7±2.4^{c}	139.3±1.9^{e}	152.2±4.6^{c}
Δ BL 3N:2N (%)	14.3	6.2	14.3	-	-	-
AGR (g day^{-1})	3.2±0.2^{a}	2.2±0.2^{d}	2.8±0.2^{b}	2.6±0.1^{c}	2.1±0.1^{e}	2.3±0.1^{d}
FCR	1.2±0.1^{b}	1.4±0.1^{c}	1.1±0.0^{a}	1.2±0.1^{b}	1.4±0.0^{c}	1.4±0.0^{c}

SR (%)	100.0±0.0[a]	93.3±5.8[c]	100.0±0.0[a]	96.7±2.9[b]	91.7±2.9[c]	98.3±2.9[ab]

Note: Δ = gain, Δ B 3N:2N = relative percentage of triploid:diploid biomass gain, BW = body weight, Δ BW 3N:2N = relative percentage of triploid:diploid body weight gain, BL = body length, Δ BL 3N:2N = relative percentage of triploid:diploid body length gain, AGR = absolute growth rate, FCR = feed conversion ratio, and SR = survival rate. Different superscripts in the same row indicates significant differences ($P < 0.05$)

Table 2. Flesh percentages of male and female both triploid and diploid of Nile tilapia fish (n = 10).

Fish group		Body weight (g)	Dressing		Edible carcass	
			Weight (g)	(%)	Weight (g)	(%)
Triploid	♂	414.1±39.2[a]	238.3±19.9[a]	57.6±1.8[b]	170.9±16.0[a]	41.3±1.4[a]
	♀	260.8±24.0[c]	154.0±13.5[c]	59.1±1.6[a]	109.4±10.8[c]	42.0±1.2[a]
Diploid	♂	332.0±29.7[b]	187.2±18.4[b]	56.4±1.6[b]	129.4±12.4[b]	39.0±1.6[b]
	♀	259.4±14.1[c]	141.0±7.8[c]	54.4±1.3[c]	98.5±6.0[d]	38.0±1.4[b]

Note: Different superscripts in the same column indicates significant differences ($P < 0.05$)

Table 3. Flesh proximate analysis of male and female both triploid and diploid of Nile tilapia fish (% dry weight) (n = 10).

Fish group		Crude Protein	Crude Lipid	Ash	Carbohydrate
Triploid	♂	85.6 ± 0.3^{ab}	5.1 ± 0.2^{b}	6.2 ± 0.2^{c}	3.2 ± 0.7^{a}
	♀	87.0 ± 1.1^{a}	5.0 ± 0.4^{b}	5.9 ± 0.0^{d}	2.2 ± 1.5^{a}
Diploid	♂	84.2 ± 1.3^{b}	5.9 ± 0.3^{a}	7.1 ± 0.0^{a}	2.8 ± 1.7^{a}
	♀	84.3 ± 1.8^{b}	5.5 ± 0.0^{a}	6.4 ± 0.3^{b}	3.8 ± 1.5^{a}

Note: Different superscripts in the same column indicates significant differences ($P < 0.05$)

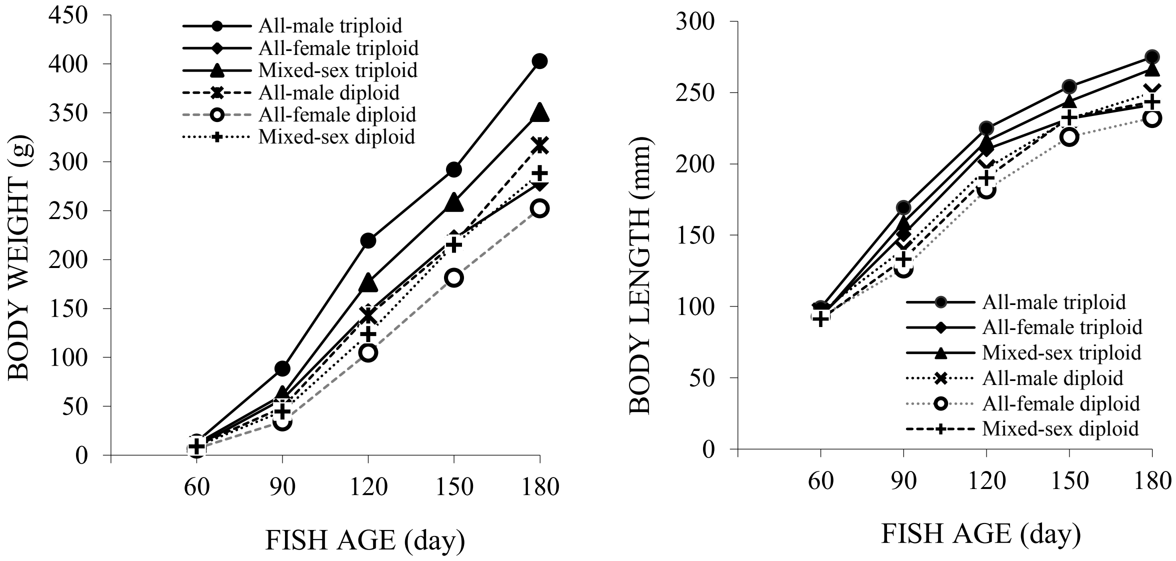

Figure 1. Body weight and body length of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period

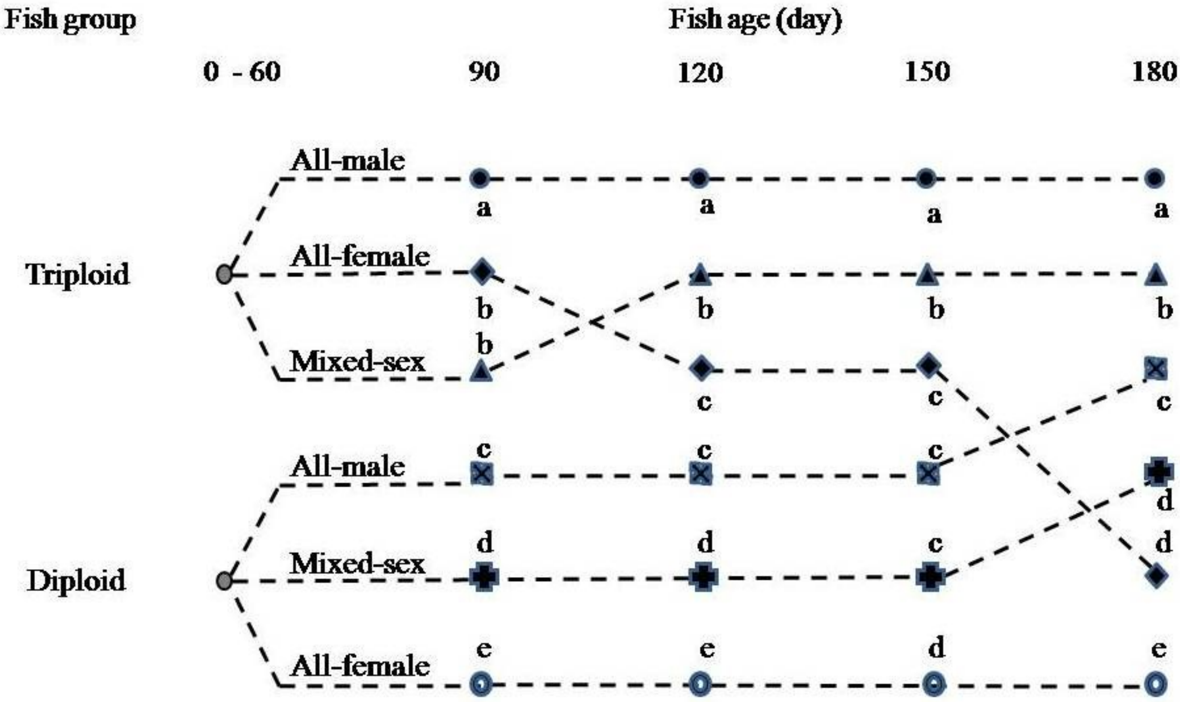

Figure 2. Schematic sequential specific growth rate (SGR) of triploid and diploid Nile tilapia fish during 4 months grow-out period. Different letters at the same fish age indicate significant differences ($P < 0.05$)

Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)

Abstract: This study aimed to compare the growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia. The triploid population was obtained through heat shock at 41 ºC for 4 minutes, 4 minutes after fertilization. Before sexing, 50 fish were reared in aquaria at a density of 1 fish L^{-1} for 2 months. After sexing, both triploid and diploid fish were grouped into all-male, all-female, and mixed-sex groups and reared in hapas at a density of 10 fish m^{-2} for 4 months. Each group was replicated three times. The highest body weight, body length, and growth rate were observed in all-male triploid, while the lowest of those parameters were obtained in all-female diploid. The highest survival rate was achieved in both all-male and mixed-sex triploids and did not significantly differ from the mixed-sex diploid ($P > 0.05$). The triploid fish had higher edible carcass percentage than diploid. The proximate analysis indicated that the crude protein content of triploid was higher than that of diploid, while the crude lipid and ash contents were lower than those of diploid ($P < 0.05$). Triploid Nile tilapia had the best growth performances, including flesh quantity and quality compared to diploid.

Keywords: Growth performance, triploid production, monosex, mixed-sex, Nile tilapia

1. Introduction

Sterile fish is beneficial in aquaculture because, in the sterile metabolism processes, the fish will reduce or even prevent the use of energy for reproduction. As a result, most of the anabolic energy will be transferred to somatic growth. Sterile fish also have the potential for a better survival rate compared to diploid fish. Devlin et al. [1] stated that the increase in the growth of fish brings substantial benefits in shortening culture period, improving the efficiency of feed utilization and the efficiency of production, and ensuring product availability. Also, culturing sterile fish is one of the best farming management in aquaculture practices, as it enables the use of the metabolism pathway to reach fast somatic tissue instead of producing either sperm or eggs in the spawning season [2].

The high ability (uncontrolled) of tilapia reproduction cause the unexpected density in the pond with varied size and slow growth, making it less commercially profitable in aquaculture. The sterilization is the best possible solution to solve the problems in the tilapia culture [3]. Lutz [4] mentioned that among future's aquaculture commodities, tilapia is a candidate fish to produce functionally sterile seeds on a large scale. The induction of triploidy is one of the methods of producing sterile fish. The culture of triploid fish could provide benefits, such as increased growth, carcass production, survival rate, and flesh quality [5-7].

The production of triploid tilapia has been developed for more than four decades, and triploidy is an effective management tool in tilapia farming in the future [8]. Triploid tilapia has small testis or ovaries, low gonad weight and high body weight, protein utilization, and protein efficiency ratio compared to diploid tilapia. Thus, farming is possibly beneficial [9]. In some cases, the growth performances of triploid tilapia were reported to be superior or equal to those of diploid tilapia [10-12].

On the other hand, some studies indicated that male tilapia has faster growth compared to female tilapia [13-15]. The production level of monosex male tilapia farming was 10% higher compared to the mixed-sex population [16,17]. Associated with presence of sexual dimorphism in terms of growth, many efforts were made to produce all-male seed population for the purpose of monosex culture, which generally can be obtained through four common methods, namely manual sexing [18] at body size of 5-7 cm, hybridization [7,19], hormonal treatments [15,20-27] or chromosome set manipulations, such as androgenesis [18,28] to produce YY supermale parent stocks [29-31].

So far, the combined effects of triploidy and growth-related sexual dimorphism superiorities in tilapia are still unknown. A strain of fish, including tilapia, also possibly influence growth performance during the culture period. Therefore, the present study tries to clarify the effect of those superiorities on growth, survival rate, flesh percentage, and proximate composition of Nile tilapia during the grow-out period.

2. Materials and methods

2.1. Experimental fish preparation

In this study, fish used was the Wanayasa strain of Nile tilapia known as NIRWANA produced through family selection program between the genetic improvement for farmed tilapia (GIFT) and the genetically enhanced tilapia (GET) in Indonesia. The broodstocks were obtained from the Tilapia and Common Carp Aquaculture Development Agency in Purwakarta, West Java, Indonesia. Artificially fertilized eggs (4 minutes after insemination) were subjected to heat shock treatment at 41 ºC for 4 minutes to produce triploid fish. This treatment was produced triploid Nile tilapia of 91-100%, as identified using the chromosome counting method prepared according to Kligerman and Bloom [32] and Mukti et al. [33]. Embryos were incubated in glass funnel in a recirculating system and diploid fish were produced using a similar procedure.

Larvae of both triploid and diploid were separately reared in 50-L aquaria at a density of 1 fish L^{-1}. A total of 10 aquaria were used for triploid and diploid fish, respectively. The 2-days-old fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Next, fish were transferred into 180-L aquaria, reared at a density of 4 fish L^{-1} and fed on a commercial diet (40% crude protein content) for 30 days. Sexing was conducted morphologically by observing the genital openings on the average fish weight of 6.5-10 g to separate males and females of both triploid and diploid fish. The sexing was also confirmed by gonad preparation and observation using the squash method with acetocarmine stain. Twenty fish from different groups, namely all-male triploid, all-female triploid, mixed-sex triploid, all-male diploid, all-female diploid, and mixed-sex diploid were respectively prepared for performance evaluation.

2.2. Performances evaluation

Previously prepared all-male, all-female, and mixed-sex of both triploid and diploid were separately transferred and reared in 2.0 m × 1.0 m × 0.7 m dimensions of floating net (mesh size of 10 mm) placed in a 20 m × 10 m × 1.5 m dimensions of concrete pond at a density of 10 fish m^{-2} with the water exchange rate of 1 L s^{-1}. On the other hand, water qualities, such as temperature, dissolved oxygen, and pH were measured every week with ranges of 27-29 ºC, 3.4-4.4 mg L^{-1}, and 6.7-7.3, respectively. Three floating nets were used as replication for each group. Firstly, fish were fed on a 1-mm-diameter commercial diet (40% crude protein content) at satiation for 30 days, then they were fed on a 3-mm-diameter commercial diet (33% crude protein content) at satiation during the last 3 months (90 days), three times a day.

In general, the maturation period of tilapia begins after 90-days-old fish. In this study, a maturation period was also observed at the 90th day of fish rearing. The gender of the fish was checked monthly. Body weight (BW), body length (BL), mortality, and feed intake data were measured every month. Biomass gain, the relative percentages of biomass, BW, and BL gains triploid compared to diploid, BW and BL gains, absolute growth rate (AGR), feed conversion ratio (FCR), and survival rate (SR) were analyzed based on data of initial and final grow-outs, except specific growth rate (SGR) was analyzed every month during 4 months grow-out of fish, while dressing, edible carcass, and proximate data of male and female both triploid and diploid fish were analyzed at the end of the experimental period.

The growth performances were calculated according to Hariati [34]. The formulas were used to calculate biomass gain (Δ), the relative percentage of triploid:diploid biomass gain, BW gain, the relative percentage of triploid:diploid BW gain, BL gain, the relative percentage of triploid:diploid BL gain, AGR, FCR, SR, and SGR, respectively, as follows:

$$\Delta \text{ Biomass (g)} = \text{Final biomass (g)} - \text{initial biomass (g)}$$

$$\Delta \text{ B 3N:2N (\%)} = \frac{\Delta \text{ biomass of triploid (g)} - \Delta \text{ biomass of diploid (g)}}{\Delta \text{ biomass of diploid (g)}} \times 100$$

$$\Delta \text{ BW (g)} = \text{Final body weight (g)} - \text{initial body weight (g)}$$

$$\Delta \text{ BW 3N:2N (\%)} = \frac{\Delta \text{ BW of triploid (g)} - \Delta \text{ BW of diploid (g)}}{\Delta \text{ BW of diploid (g)}} \times 100$$

$$\Delta \text{ BL (mm)} = \text{Final body length (mm)} - \text{initial body length (mm)}$$

$$\Delta \text{ BL 3N:2N (\%)} = \frac{\Delta \text{ BL of triploid (mm)} - \Delta \text{ BL of diploid (mm)}}{\Delta \text{ BL of diploid (mm)}} \times 100$$

$$\text{AGR (g day}^{-1}\text{)} = \frac{\text{Final body weight (g)} - \text{initial body weight (g)}}{\text{Long time of rearing (day)}}$$

$$\text{FCR} = \frac{\text{Feed consumed by fish (g)}}{\Delta \text{ body weight of fish (g)}}$$

$$\text{SR (\%)} = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\text{Ln final body weight} - \text{Ln initial body weight}}{\text{Long time of rearing (day)}} \times 100$$

The dressing is a piece of fish's body without a head, fins, scales, and internal organs, while the edible carcass is a cut of the right and the left sides of the fish's body. The dressing and edible carcass data were determined according to Buchtova et al. [35] based on ten samples from males and females both triploid and diploid, respectively. The dressing and the edible carcass percentages were calculated by formulas, respectively:

$$\text{Dressing (\%)} = \frac{\text{Dressing weight of fish}}{\text{Body weight of fish}} \times 100$$

$$\text{Edible carcass (\%)} = \frac{\text{Edible carcass weight of fish}}{\text{Body weight of fish}} \times 100$$

Increase of triploid dressing percentage (DP) and edible carcass percentage (ECP) compared to diploid was calculated using the relative percentages of triploid:diploid dressing and edible carcass formulas, respectively, as follows:

$$\Delta \text{ Dressing 3N:2N (\%)} = \frac{\text{DP of triploid (\%)} - \text{DP of diploid (\%)}}{\text{DP of diploid (\%)}} \times 100$$

$$\Delta \text{ Edible carcass 3N:2N (\%)} = \frac{\text{ECP of triploid (\%)} - \text{ECP of diploid (\%)}}{\text{ECP of diploid (\%)}} \times 100$$

In addition, flesh proximate analysis of fish (crude protein, crude lipid, ash, and carbohydrate contents) was evaluated according to AOAC protocol [36] based on ten samples from male and female both triploid and diploid, respectively.

2.3. Statistical analysis

Data on growth performances (biomass gain, body weight and body length gains, AGR, and SGR), FCR, SR, and flesh percentage (dressing and edible carcass percentages), and proximate content were statistically analyzed using the analysis of variance (ANOVA) with SPSS ver.10 software. Duncan's multiple range test was followed by the ANOVA test with a confidence level of 95%.

3. Results

3.1. Growth performance, survival rate, and feed conversion ratio

The growth performances of the tested fish groups are shown in Table 1. The results showed that the growth of triploid fish was significantly higher ($P < 0.05$) compared to that of diploid. The biomass gains (Δ B 3N:2N) of all-male, all-female, and mixed-sex triploids fish were 31.3, 11.4, and 23.4% higher than those of diploid, respectively. A similar pattern was found in body weight gain (Δ BW 3N:2N) and body length gain (Δ BL 3N:2N). The highest values of body weight and length gains (26.8 and 14.3%, respectively) were observed in all-male triploid, followed by mixed-sex triploid (21.4 and 14.3%, respectively), while the lowest values (9.6 and 6.2%, respectively) were seen in all-female triploid. Furthermore, all-female diploid fish significantly showed the most inferior growth performance compared to other groups.

All-male triploid had the highest absolute growth rate (AGR) than other groups, followed by mixed-sex triploid, then all-male and all-female diploids. Meanwhile, the mixed-sex triploid had the best feed conversion ratio, followed by all-male triploid and diploid. The survival rates of all-male and mixed-sex triploids and mixed-sex diploid were higher compared to other groups, as shown in Table 1.

Figure 1 shows the monthly body weight and body length recorded during the 4 months grow-out period. In general, triploid grew faster than diploid, and all-male triploid showed the highest growth rate, while all-female diploid showed the lowest growth rate.

In this study, it was observed that in both triploid and diploid fish, males grew faster than females during the experiment. In triploid and diploid groups, the biomass gains of the male were 55.5 and 31.9% higher than those of females, respectively. Before the maturation period, the average body weight of triploid and diploid males was 16.6 and 10.7 g bigger than those of triploid and diploid females, respectively. Meanwhile, during the maturation period, the average body weight of triploid and diploid males was 103.3 and 50.5 g bigger than those of triploid and diploid females, respectively. These results showed that the role of the sexual dimorphism on growth in Nile tilapia had a similar pattern with the role of the ploidy level, the effects of which were highly significant during the maturation period.

All-female and mixed-sex triploids groups showed a similar growth rate at the 90th day (Figure 2). The mixed-sex triploid group has higher specific growth rate (SGR) than other sex groups at the 120th to 180th day, while the all-female triploid group has similar SGR as an all-male diploid group at the 120th day. On the other hand, all-female triploid and all-male and mixed-sex diploids groups have similar SGR at the 150th day. Meanwhile, the all-female triploid group has a similar SGR as the mixed-sex diploid group at the 180th day (Figure 2).

3.2. Flesh percentage and proximate composition

The edible carcass percentages of male and female triploids were higher than those of diploids. The highest and lowest dressing percentages were found in triploid and diploid females, respectively ($P < 0.05$). The increase in dressing and edible carcass percentages of female triploid were 8.6 and 10.5% higher than those of female diploid, respectively. Meanwhile, the increase in dressing and edible carcass percentages of male triploid were 2.1 and 5.9% higher than those of the diploids, respectively (Table 2).

Flesh proximate analysis of triploid and diploid fish is shown in Table 3. The crude protein content of female triploid was similar to that of male triploid, however, it was higher than that of diploid fish ($P < 0.05$). On the other hand, crude lipid and ash contents of male and female triploids were lower than diploids. There were no significant differences in carbohydrate content between triploid and diploid fish.

4. Discussion

This study revealed that ploidy level and sexual dimorphism play essential roles in Nile tilapia growth performance. The high growth of male triploid and low growth of female diploid indicated that both ploidy level and sexual dimorphism significantly affected Nile tilapia growth (Table 1 and Figures 1 and 2).

Tave [37] reported that triploidization leads to an increase in sterility and growth. A cell size of triploid is larger than diploid, and energy for gamete production is reduced or inhibited. In most cases, triploid showed heavier body size and faster growth than diploid in common carp (*Cyprinus carpio*) [38], African mud catfish (*Clarias gariepinus*) [39], Chinese catfish (*C. fuscus*) [40], and Atlantic salmon (*Salmo salar*) [41]. Besides, the performances of triploid fish were not only species and age-dependent but also depended on the experimental conditions and the interactions between the environment and genetics [7]. The individual body size of triploid was more significant due to the larger cell size compared to diploid [42]. However, Aliah et al. [43] reported that the cell size was not correlated with the organ size in sticklebacks (*Gasterosteus aculeatus*). Furthermore, in 2-3 month-old sunshine bass (*Morone* spp.), diploid grew faster compared to triploid [44].

The increase in triploid growth is due to the influence of sterility, diverting energy (nutrient) for somatic growth rather than gonadal development and sexual activity [14]. Most studies concluded that the significant difference in growth rate between triploid and diploid fish occurred during the maturation period in fish such as turbot (*Scophthalmus maximus*) [45] and European sea bass (*Dicentrarchus labrax*) [46]. In this study, it was found that the growth difference (30.0%) between triploid and diploid fish already occurred before (≤ 90-days-old) and during the maturation period (90- to 180-day-old). Also, the growth of triploid showed more significant differences compared to diploid (39.3%). A similar phenomenon has been reported in fancy carp (*C. carpio*) [47].

The role of sexual dimorphism in growth in tilapia has been revealed in the last three decades. Male tilapia grew faster compared to females, so the all-male monosex culture in this species is worldwide applied. Similar cases were found in catfish (*C. gariepinus*) [48] and crucian carp (*Carassius auratus*) [49].

The comparison of the growth performance among the six groups showed that all-male triploid and all-female diploid fish grew faster and lower, respectively than the fish in other groups during the experiment. The interaction effect between triploidy and sexual dimorphism in growth was not significant among all-female triploid, all-male diploid, and mixed-sex diploid groups at the 120th to 150th day. In the same groups, all-male diploid grew faster than the others and the interaction effect between triploidy and sexual dimorphism on growth was not significant among all-female triploid and mixed-sex diploid at the 180th day (Figure 2). This phenomenon seemed to be species-specific as found in rainbow trout (*Oncorhynchus mykiss*) by Tabata et al. [50], Mozambique tilapia (*O. mossambicus*) by Varadaraj and Pandian [51] and European sea bass by Felip et al. [52]. Those authors reported that female triploid grew faster than either male triploid, male and female diploids or mixed-sex diploid.

The lowest growth was observed in all-female diploid looked as if the female diploid went through rapid reproductive development and sexual maturity. So, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth. In this study, it was recorded that at the 120th day, the majority of female diploid began to spawn and incubate either fertilized or unfertilized eggs in the mouth. This aspect generally allows the female to not feed during eggs incubation for 15 days until larvae can swim freely, as reported by Byamungu et al. [53]. In other words, the role of the ploidy level in growth during the maturation period was significantly higher than that before the maturation period. These results also revealed that a high body weight gain in male and female triploid during maturation period seemed to be due to the sterility of triploid fish and reproductive activity of diploid fish.

In this study, triploid fish had higher flesh percentages compared to diploid, and female triploid also had higher flesh percentages. Similar results were reported in gilthead sea bream (*Sparus aurata*) [54] and rainbow trout [55]. However, in common carp [56] up to the size of 400 g, the dressing weight of triploid was not significantly different from that of diploid. The results of this study indicated that higher flesh percentages of female triploid compared to male triploid was because the female was more sterile than male, while the higher flesh percentages in triploid compared to diploid seemed correlated with normal in diploid and reducing in triploid through gonadal developments.

Triploid Nile tilapia tends to be high in crude protein and low in crude lipid and ash compared to diploid. In terms of sex, male and female fish from both triploid and diploid show the same crude protein, crude lipid and carbohydrates contents, while the ash content was significantly different. This result showed that triploidy in Nile tilapia affects flesh quality, especially crude lipid and ash contents. This result indicated that as well as a study conducted by other researchers [5,6,11]. Further study is needed to gather more valuable information.

The interaction effect between triploidy and sexual dimorphism strongly related to growth had a positive contribution to production performance, especially during the maturation period. Based on the examination of various aspects related to production, the result revealed that all-male triploid Nile tilapia culture has the potential to be developed. Hence, in the future, an applicable method for mass all-male triploid seed production should be considered. One of the possible strategic efforts is how to produce supermale tetraploid as parent stock by combining the chromosome set and hormonal manipulations.

References

1. Devlin R, Biagi CA, Yesaki TY. Growth, viability and genetic characteristics of GH transgenic Coho salmon strains. Aquaculture 2004; 236 (1-4): 607-632. doi:10.1016/j.aquaculture.2004.02.026

2. Galli L. Genetic Modification in Aquaculture: A Review of Potential Benefits and Risks. Bureau of Rural Sciences, Australia: Canberra; 2002.

3. Pradeep PJ, Srijaya TC, Jose D, Papini A, Hassan A, et al. Identification of diploid and triploid red tilapia by using erythrocyte indices. Caryologia 2011; 64 (4): 485-492. doi:10.1080/00087114.2011.10589816

4. Lutz CG. Practical Genetics for Aquaculture. Fishing News Books, Oxford: Blackwell Science; 2001.

5. Felip A, Zanuy S, Carrillo M, Piferrer F. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 2001; 111 (1-3): 175-195.

6. Melamed P, Gong Z, Fletcher G, Hew CL. The potential impact of modern biotechnology on fish aquaculture. Aquaculture 2002; 204 (3-4): 255-269. doi:10.1016/S0044-8486(01)00838-9

7. Dunham RA. Aquaculture and Fisheries Biotechnology: Genetic Approaches. Cambridge: CABI Publishing; 2004.

8. Pradeep PJ, Srijaya TC, Bahuleyan A, Papini A. Can sterility through triploidy induction make an impact on Tilapia industry? International Journal of Aquatic Science 2012; 3 (2): 89-96.

9. Pechsiri J, Yakupitiyage A. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 2005; 36 (1): 45-51. doi:10.1111/j.1365-2109.2004.01182.x

10. Mol K, Byamungu N, Cuisset B, Yaron Z, Ofir M, et al. Hormonal profile of growing male and female diploids and triploids of the blue tilapia (*Oreochromis*

aureus) reared in intensive culture. Fish Physiology and Biochemical 1994; 13 (3): 209-218. doi:10.1007/BF00004359

11. Hussain MG, Rao GPS, Humayun NM, Randall CF, Penman DJ, et al. Comparative performance of growth, biochemical composition, and endocrine profiles in diploid and triploid tilapia (*Oreochromis niloticus* L.). Aquaculture 1995; 138 (1-4): 87-97. doi:10.1016/0044-8486(95)01079-3
12. Puckhaber B, Horstgen-Schwark G. Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). In: ICLARM Conference Proceedings of The Third International Symposium on Tilapia in Aquaculture. Manila; 1996. pp. 377-382.
13. Bhatta S, Iwai T, Miura T, Huguchi M, Maugars G, et al. Differences between male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). Kathmandu University Journal of Science, Engineering, and Technology 2012; 8 (II): 57-65. doi:10.3126/kuset.v8i2.7326
14. Pradeep PJ, Srijaya TC, Papini A, Chatterji AK. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture 2012; 344-349: 181-187. doi:10.1016/j.aquaculture.2012.03.006
15. Fuentes-Silva C, Soto-Zarazua GM, Torres-Pacheco I, Flores-Rangel A. Male tilapia production techniques: a mini-review. African Journal of Biotechnology 2013; 12 (36): 5496-5502. doi:10.5897/AJB11.4119
16. Nguyen CD, David CL. The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis*

niloticus) in Northern Vietnam. Aquaculture 2000; 184 (3-4): 221-231. doi:10.1016/S0044-8486(99)00329-4

17. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. Journal of Endocrinology 2001; 170 (1): 27-38. doi:10.1677/joe.0.1700027
18. Cnaani A, Levavi-Sivan B. Sexual development in fish: practical applications for aquaculture. Sex Development 2009; 3 (2-3): 164-175. doi:10.1159/000223080
19. Bartley D, Rana K, Immink A. The use of interspecific hybrids in aquaculture and fisheries. Review Fish Biology and Fisheries 2001; 10 (3): 325-337. doi:10.1023/A:1016691725361
20. Popma TJ, Green BW. Sex Reversal of Tilapia in Earthen Ponds. Aquaculture Production Manual, Research and Development Series No. 35, International Center for Aquaculture, Auburn University, USA: Alabama; 1991.
21. Pandian TJ, Sheela SG. Hormonal induction of sex reversal in fish. Aquaculture 1995; 138 (1-4): 1-22. doi:10.1016/0044-8486(95)01075-0
22. Mukti AT. Optimation of 17α-methyltestosterone synthetic hormone dose and immersion duration in larvae on the success of Nile tilapia (*Oreochromis* sp.) sex reversal. BSc, Faculty of Fisheries, Brawijaya University, Malang, Indonesia, 1998 (in Indonesian).
23. Romerio MP, Fencrich-Verani CSN, Santo De-Copmus BE, Pasilva AS. Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasil Zoology 2000; 29 (3): 654-659. doi:10.1590/S1516-35982000000300003

24. Mukti AT, Priyambodo B, Rustidja, Widodo MS. Optimization of both 17α-methyltestosterone synthetic hormone dosage and dipping duration of Nile tilapia (*Oreochromis* sp.) larvae on sex reversal efficacy. BIOSAIN Journal of Life Science 2002; 2 (1): 1-8 (in Indonesian with an abstract in English).

25. Mohamed AH, Traifalgar RFM, Serrano Jr. AE, Peralta JP, Pedroso FL. Dietary administration of dehydroepiandrosterone hormone influences the sex differentiation of hybrid red Tilapia (*O. niloticus* × *O. mossambicus*) larvae. Journal of Fisheries and Aquatic Science 2012; 7 (6): 447-453. doi:10.3923/jfas.2012.447.453

26. Beaven U, Muposhi V. Aspects of a monosex population of (*Oreochromis niloticus*) fingerling produced using 17-α methyltestosterone hormone. Journal of Aquaculture Research and Development 2012; 3 (3): 132. doi:10.4172/2155-9546.1000132

27. Dagne A, Degefu F, Lakew A. Comparative growth performance of monosex and mixed-sex Nile tilapia (*Oreochromis niloticus* L.) in pond culture system at Sebeta, Ethiopian. International Journal of Aquaculture 2013; 3 (7): 30-34. doi:10.5376/ija.2013.03.0007

28. Ezaz MT, Myers JM, Powell SF, McAndrew BJ, Penman DJ. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 2004; 232 (1-4): 205-214. doi:10.1016/j.aquaculture.2003.08.001

29. Muller-Belecke A, Horstgen-Schwark G. A YY-male (*Oreochromis niloticus*) strain developed from an exceptional mitotic gynogenetic male and growth

performance testing of genetically all-male progenies. Aquaculture Research 2007; 38 (7): 773-775. doi:10.1111/j.1365-2109.2007.01712.x

30. Aliah RS, Sumantadinata K, Maskur, Naim S. GESIT tilapia: Indonesia's genetic supermales. Global Aquaculture Advocate, May/June 2010; 36-37.

31. Turra EM, Oliveira DAA, Teixeira EA, Luz RK, Prado SA, et al. Reproduction control in Nile tilapia (*Oreochromis niloticus*) by sexual and chromosome set manipulation. Revista Brasil de Reproduction Animal, Belo Horizonte 2010; 34 (1): 21-28.

32. Kligerman AD, Bloom SE. Rapid chromosome preparation from solid tissues of fish. Journal of Fisheries Research Board Canada 1977; 34: 266-269. doi:10.1139/f77-039

33. Mukti AT, Carman O, Alimuddin, Zairin Jr. M. A rapid chromosome preparation technique without metaphase arrest for ploidy determination in Nile tilapia (*Oreochromis niloticus*). Caryologia 2016; 6 (2): 175-180. doi:10.1080/00087114.2016.1152112

34. Hariati AM. Fish Feed. Nuffic/Unibraw/Luw/Fish Fisheries Project, Malang: Brawijaya University; 1989 (in Indonesian).

35. Buchtova H, Svobodova Z, Kocour M, Velisek J. Evaluation of the dressing percentage of 3-year-old experimental scaly crossbreds of the common carp *Cyprinus carpio* (Linnaeus, 1758) in relation to sex. Acta Veterinary Brno 2006; 75 (1): 123-132. doi:10.2754/avb200675010123

36. AOAC [Association of Official Analytical Chemists]. Official Methods of Analysis. 18th ed. Washington: Association of Official Analytical Chemists Inc.; 2005.

37. Tave D. Genetics for Fish Hatchery Managers. Connecticut: Avi Publishing; 1993.

38. Mukti AT, Rustidja, Sumitro SB, Djati MS. Polyploidization of common carp (*Cyprinus carpio* L.). BIOSAIN Journal of Life Science 2001; 1 (1): 111-123 (in Indonesian with an abstract in English).

39. Lawson EO, Ishola HA. Effects of cold shock treatment on the survival of fertilized eggs and growth performance of the larvae of African mud catfish *Clarias gariepinus* (Burchell, 1822). Research Journal of Fisheries and Hydrobiology 2010; 5 (2): 85-91.

40. Qin JG, Fast AW, Ako H. Grow-out performance of diploid and triploid Chinese catfish (*Clarias fuscus*). Aquaculture 1998; 166 (3-4): 247-258. doi:10.1016/S0044-8486(98)00287-7

41. Burke HA, Sacobie CFD, Lall SP, Benfey TJ. The effect of triploidy on juvenile Atlantic salmon (*Salmo salar*) response to varying levels of dietary phosphorus. Aquaculture 2010; 306 (1-4): 295-301. doi:10.1016/j.aquaculture.2010.05.002

42. Piferrer F, Beaumont A, Falguière J-C, Flajšhans M, Haffray P, et al. Polyploid fish and shellfish: production, biology, and applications to aquaculture for performance improvement and genetic containment. Aquaculture 2009; 293 (3-4): 125-156. doi:10.1016/j.aquaculture.2009.04.036

43. Aliah RS, Yamaoka K, Inada Y, Taniguchi N. Effects of triploidy on tissue structure of some organs in ayu. Bulletin Japan Society Science Fisheries 1990; 56 (4): 569-575. doi:10.2331/suisan.56.569

44. Kerby JH, Eversona JM, Harrell RM, Geiger JG, Starling CC, et al. Performance comparisons between diploid and triploid sunshine bass in freshwater ponds. Aquaculture 2002; 211 (1-4): 91-108. doi:10.1016/S0044-8486(02)00009-1

45. Cal RM, Vidal S, Gomez C, Ivarez-Blazquez BA, Martinez P, et al. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). Aquaculture 2006; 251 (1): 99-108. doi:10.1016/j.aquaculture.2005.05.010

46. Felip A, Piferrer F, Zanuy S, Carrillo M. Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons. Journal of Fish Biology 2001; 58 (1): 76-88. doi:10.1111/j.1095-8649.2001.tb00500.x

47. Taniguchi N, Kijima A, Tamura T, Takegami K, Yamasaki I. Color, growth, and maturation in ploidy-manipulated fancy carp. Aquaculture 1986; 57 (1-4): 321-328. doi:10.1016/0044-8486(86)90210-3

48. Achegbulu CE, Okonji VA, Obi A. Growth and economic performance of diploid and triploid African catfish (*Clarias gariepinus*) in outdoor concrete tanks. International Journal of Genetics 2013; 3 (1): 01-06. doi:10.5829/idosi.ijg.2013.3.1.738

49. Chen S, Wang J, Liu SJ, Qin QB, Xiao J, et al. Biological characteristics of an improved triploid crucian carp. Science China Series C: Life Science 2009; 52 (8): 733-738. doi:10.1007/s11427-009-0079-3

50. Tabata YA, Rigolino MG, Tsukamoto RY. Production of all-female triploid rainbow trout (*Oncorhynchus mykiss*) [Pisces, Salmonidae]. III. Growth up to first sexual maturation. Boletim do Instituto de Pesca Sao Paulo 1999; 25: 67-76.

51. Varadaraj K, Pandian TJ. Production of all-female sterile-triploid (*Oreochromis mossambicus*). Aquaculture 1990; 84 (2): 117-123. doi:10.1016/0044-8486(90)90342-K

52. Felip A, Carrillo M, Zanuy S. Older triploid fish retain impaired reproductive endocrinology in the European sea bass (*Dicentrarchus labrax*). Journal of Fish Biology 2009; 75 (10): 2657-2669. doi:10.1111/j.1095-8649.2009.02458.x

53. Byamungu N, Darras VM, Kuhn ER. Growth of heat-shock induced triploids of blue tilapia (*Oreochromis aureus*) reared in tanks and in ponds in Eastern Congo: feeding regimes and compensatory growth response of triploid females. Aquaculture 2001; 198 (1-2): 109-122. doi:10.1016/S0044-8486(00)00605-0

54. Haffray P, Bruant J-S, Facqueur J-M, Fostier A. Gonad development, growth, survival and quality traits in triploids of the protandrous hermaphrodite gilthead sea bream (*Sparus aurata* L.). Aquaculture 2005; 247 (1-4): 107-117. doi:10.1016/j.aquaculture.2005.02.037

55. Werner C, Poontawee K, Mueller-Belecke A, Horstgen-Schwark G, Wicke M. Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. Archiv Tierzucht 2008; 51 (1): 71-83. doi:10.5194/aab-51-71-2008

56. Basavaraju Y, Mair GC, Kumar HMM, Kumar SP, Keshavappa GY, et al. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp (*Cyprinus carpio*) in Karnataka, India. Aquaculture 2002; 204 (3-4): 407-418. doi:10.1016/S0044-8486(01)00827-4

Table 1. The growth, survival rate, and feed conversion ratio performances of sex-grouped triploid and diploid Nile tilapia fish during 4 months grow-out period (n = 20).

Parameter	Fish groups					
	Triploid			Diploid		
	All-male	All-female	Mixed-sex	All-male	All-female	Mixed-sex
Initial biomass (g)	278.6±5.2	190.0±8.3	236.2±6.0	205.0±8.9	136.0±8.8	183.4±5.8
Final biomass (g)	8056.7±405.5	5193.3±445.6	7013.3±551.4	6130.0±366.6	4626.7±277.6	5676.7±465.0
Δ Biomass (g)	7778.1±404.3^{a}	5003.0 ±437.9^{e}	6777.1±548.9^{b}	5925.0±363.5^{c}	4490.7±284.9^{f}	5493.2±462.9^{d}
Δ B 3N:2N (%)	31.3	11.4	23.4	-	-	-
Initial BW (g)	13.9±0.3	9.5±0.4	11.8±0.3	10.3±0.4	6.8±0.4	9.2±0.3
Final BW (g)	402.8±20.3	278.5±23.2	350.7±27.6	317.0±13.5	252.3±10.2	288.3±15.5
Δ BW (g)	388.9±20.2^{a}	269.0±22.8^{d}	338.9±27.4^{b}	306.7±13.6^{c}	245.5±10.7^{e}	279.2±15.3^{d}
Δ BW 3N:2N (%)	26.8	9.6	21.4	-	-	-
Initial BL (mm)	99.2±0.0	93.3±0.0	92.5±0.0	96.3±0.0	92.8±0.0	91.2±0.0
Final BL (mm)	274.5±2.1	241.3±6.7	266.5±5.6	250.0±2.4	232.2±1.9	243.4±4.6
Δ BL (mm)	175.7±2.1^{a}	147.9±6.7^{d}	174.0±5.6^{b}	153.7±2.4^{c}	139.3±1.9^{e}	152.2±4.6^{c}
Δ BL 3N:2N (%)	14.3	6.2	14.3	-	-	-
AGR (g day^{-1})	3.2±0.2^{a}	2.2±0.2^{d}	2.8±0.2^{b}	2.6±0.1^{c}	2.1±0.1^{e}	2.3±0.1^{d}
FCR	1.2±0.1^{b}	1.4±0.1^{c}	1.1±0.0^{a}	1.2±0.1^{b}	1.4±0.0^{c}	1.4±0.0^{c}

SR (%)	100.0±0.0^{a}	93.3±5.8^{c}	100.0±0.0^{a}	96.7±2.9^{b}	91.7±2.9^{c}	98.3±2.9^{ab}

Note: Δ = gain, Δ B 3N:2N = relative percentage of triploid:diploid biomass gain, BW = body weight, Δ BW 3N:2N = relative percentage of triploid:diploid body weight gain, BL = body length, Δ BL 3N:2N = relative percentage of triploid:diploid body length gain, AGR = absolute growth rate, FCR = feed conversion ratio, and SR = survival rate. Different superscripts in the same row indicates significant differences ($P < 0.05$)

Table 2. Flesh percentages of male and female both triploid and diploid of Nile tilapia fish (n = 10).

Fish group		Body weight (g)	Dressing		Edible carcass	
			Weight (g)	(%)	Weight (g)	(%)
Triploid	♂	414.1±39.2^{a}	238.3±19.9^{a}	57.6±1.8^{b}	170.9±16.0^{a}	41.3±1.4^{a}
	♀	260.8±24.0^{c}	154.0±13.5^{c}	59.1±1.6^{a}	109.4±10.8^{c}	42.0±1.2^{a}
Diploid	♂	332.0±29.7^{b}	187.2±18.4^{b}	56.4±1.6^{b}	129.4±12.4^{b}	39.0±1.6^{b}
	♀	259.4±14.1^{c}	141.0±7.8^{c}	54.4±1.3^{c}	98.5±6.0^{d}	38.0±1.4^{b}

Note: Different superscripts in the same column indicates significant differences ($P < 0.05$)

Table 3. Flesh proximate analysis of male and female both triploid and diploid of Nile tilapia fish (% dry weight) (n = 10).

Fish group		Crude Protein	Crude Lipid	Ash	Carbohydrate
Triploid	♂	85.6±0.3[ab]	5.1±0.2[b]	6.2±0.2[c]	3.2±0.7[a]
	♀	87.0±1.1[a]	5.0±0.4[b]	5.9±0.0[d]	2.2±1.5[a]
Diploid	♂	84.2±1.3[b]	5.9±0.3[a]	7.1±0.0[a]	2.8±1.7[a]
	♀	84.3±1.8[b]	5.5±0.0[a]	6.4±0.3[b]	3.8±1.5[a]

Note: Different superscripts in the same column indicates significant differences ($P < 0.05$)

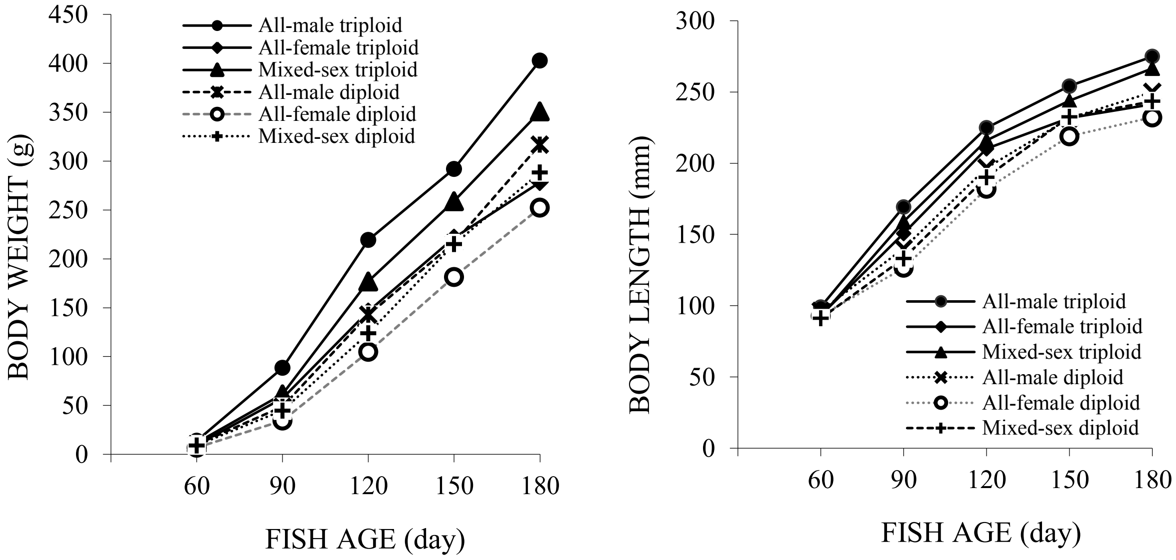

Figure 1. Body weight and body length of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4 months grow-out period

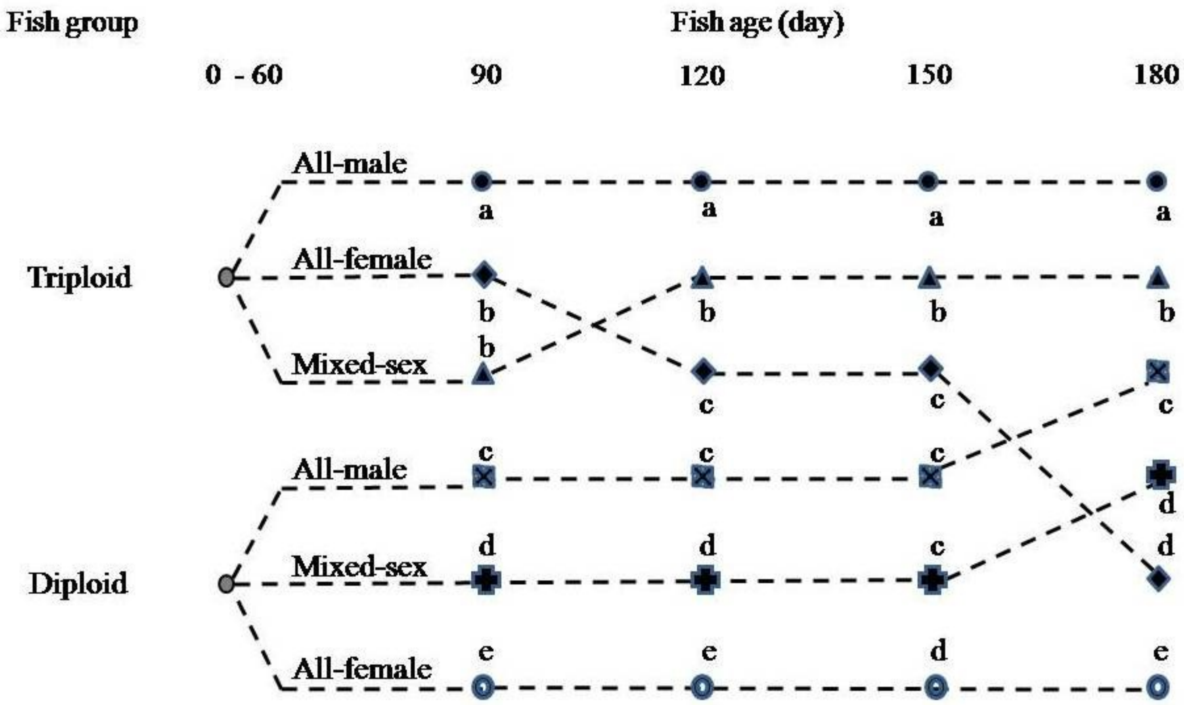

Figure 2. Schematic sequential specific growth rate (SGR) of triploid and diploid Nile tilapia fish during 4 months grow-out period. Different letters at the same fish age indicate significant differences ($P < 0.05$)

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Senin, 2 Desember 2019 19.45 GMT+7

Dear AKHMAD MUKTI

Your manuscript has been evaluated and has been found to be lacking in the respects stated below. You are requested to correct these deficiencies and re-submit your manuscript within 30 days so that its processing may begin immediately.

Deficiencies:

1. References not written according to the instructions
2. Punctuation in References not compatible with the instructions
3. Other reasons (Look at technical comments)

Technical Comments: Until your manuscript complies with all of the journal's rules it will not be processed further.
-Do not abbreviate names of journals in the end reference list. Names of journals must be written out in full
-In the end reference list, et al. must be used after the first 5 authors of a publication. Give only the names of the first 5 authors, and then et al.
-In the references section use a hyphen between page numbers and not an en dash, e.g., 114-119 and not 114?119.
-You must include a space between the volume and issue numbers in the end reference list. Example: 2012; 37 (15): 48-67.

We thank you for your interest in our journal.

Yours sincerely,

YÜCEL UYAR
Journal Administrator

Article Title: Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (Oreochromis niloticus)
Article Code Number: VET-1905-79
Web address: http://online.journals.tubitak.gov.tr

NOTE: To resubmit your manuscript, log onto the online system as corresponding author and find your manuscript in the 'Manuscripts Sent Back to Author Because of Deficiencies' list. Then you can resubmit your manuscript. Please do not use 'Submit New Manuscript' link for resubmission.

Dear
Editor-in-Chief of Turkish Journal of Veterinary and Aquatic Sciences and Reviewers

Thanks for the corrections and suggestions that have been given to our paper. Authors responses on corrections and suggestions of reviewers have mentioned in the article with blue-colored words or sentences.

For Reviewer 2

1. Reviewer comment: Title; To long and confusing with three “and”. Could be shortened.

   Authors response: We have revised the Title of article; page 1, line 1-2: “Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)”

2. Reviewer comment: Abstract; Give this rate with fish/lt or mentioned the volume of aquarium in advance in paranthesis..

   Authors response: We have revised and mentioned word in the Abstract of article; page 1, line 7: “... at a density of 1 fish L^{-1} for 2 months.”

3. Reviewer comment: Abstract; If significant used give P value.

   Authors response: We have revised and mentioned word in the Abstract of article; page 1, line 12-17 “($P > 0.05$).”

4. Reviewer comment: Introduction; Change “tests” to “studies”.

   Authors response: We have revised and mentioned word in the Abstract of article; page 3, line 3 “... some studies indicated ...”

5. Reviewer comment: Materials and methods; Which strain was used? Or both mixed? Since the study was about growing condition, it should be only one strain GIFT or GET.

   Authors response: In this study, we used the Wanayasa strain of tilapia known as NIRWANA. NIRWANA fish is a strain of tilapia obtained from crossing through a family selection program between GIFT and GET. So it can be interpreted that NIRWANA fish is the offspring of a cross between GIFT and GET.

   We have also mentioned sentences about NIRWANA strain of tilapia in the Materials and methods of article; page 3, line 20-22: “In this study, fish used was the Wanayasa strain of Nile tilapia known as NIRWANA produced through family selection program between the genetic improvement for farmed tilapia (GIFT) and the genetically enhanced tilapia (GET) in Indonesia.”

6. Reviewer comment: Materials and methods; Triploid?

   Authors response: Yes triploid Nile tilapia. We have also mentioned sentences in the Materials and methods of article; page 4, line 2-3 “This treatment was produced triploid Nile tilapia of 91-100%, as identified using ...”

7. Reviewer comment: Materials and methods; In total 15+30 days = 45 days (1.5 months) but Figure 1 starts from 60 days (2 months)?.

   Authors response: In this study, actually the total age of fish used when starting grow-out using sex group treatment is 60 days (2 months): When larvae aged 2 days, fish were fed of *Moina* for 3 days + tubificid worms for 10 days + commercial feed for 15 days (= 30 days): 2+3+10+15 days = 30 days (1 month), so 30 days (1 month) + 30 days (1 month) = 60 days (2 months).

   We have also mentioned sentences in the Materials and methods of article; page 4, line 9-12: "The 2-days-old fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Next, fish were transferred into 180-L aquaria, reared at a density of 4 fish L^{-1} and fed on a commercial diet (40% crude protein content) for 30 days."

8. Reviewer comment: Materials and methods; 2 m x 1 m x 0.7 m dimensions or 1.4 m^3 floating net.

   Authors response: We have revised and mentioned sentences in the Materials and methods of article; page 4, line 22-23 "... reared in 2.0 m × 1.0 m × 0.7 m dimensions of floating net (mesh size of 10 mm) placed in a 20 m × 10 m × 1.5 m dimensions of concrete pond ..."

9. Reviewer comment: Materials and methods; What is the water exchange rate and water quality parameters in ponds? DO, pH, temperature etc?

   Authors response: We have mentioned sentences in the Materials and methods of article; pages 4 and 5, line 24 and line 1-2, respectively: "... with the water exchange rate of 1 L s^{-1}. On the other hand, water qualities, such as temperature, dissolved oxygen, and pH were measured every week with ranges of 27-29 ºC, 3.4-4.4, and 6.7-7.3, respectively."

10. Reviewer comment: Materials and methods; Graphs shows 4 months. And in Results were given by 4 months?

    Authors response: Yes, 4 months: 1 month + 3 months = 4 months (120 days). We have also mentioned sentences in the Materials and methods of article; page 5, line 3-6: "Firstly, fish were fed on a 1-mm-diameter commercial diet (40% crude protein content) ad libitum for 30 days, then they were fed on a 3-mm-diameter commercial diet (33% crude protein content) ad libitum during the last 3 months (90 days), three times a day."

11. Reviewer comment: Materials and methods; In the Table 1, AGR, FCR and K were seen… Please put their formulas in M&M or cite a research you used the formulas from..

    Authors response: We have revised and mentioned sentences in the Materials and methods of article; pages 5 and 6, line 17-22 and line 1, respectively "The formulas were used to calculate absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), respectively, as follows:.."

12. Reviewer comment: Results; Add body weight and length gains.

    Authors response: We have revised and mentioned sentences in the Results of article; page 6, line 10: "The highest values of body weight and length gains ..."

13. Reviewer comment: Results; Need a bit revision it is 21.4 at mixed sex group… this makes confusion…

    Authors response: We have mentioned sentences in the Results of article; pages 6, line 11-12: "... mixed-sex triploid (21.4 and 14.3%, respectively),..."

14. Reviewer comment: Results; No need duplication. It would be explained below already.

    Authors response: Thank you for the correction from the reviewer. We have revised and deleted sentences in the Results of article; page 6, line 15.

15. Reviewer comment: Results; In the Table 1, Where is the condition factor? since you give FCR , no need to put Feed consumption in Table. And if you do not use K in results, dont use in Table.

    Authors response: We have revised and deleted parameter of condition factor and feed consumption in the Table 1 of article; page 20.

16. Reviewer comment: Results; Where these come from? Didn't get it? When was the maturation period. If you record kind of data and use t in results put the details in M&M pls.

    Authors response: Figure 1 shows that the difference in body weight between triploid and diploid tilapia, both male and female. The value obtained is a calculation in increasing body weight between male and female triploids and between male and female diploids, both before and during the maturation period, respectively. In general, the maturation period of Nile tilapia occurs after the 90-days-old fish. Therefore, the calculation before maturation period was conducted at the 90^{th} day, while the calculation during maturation period was conducted at the 180^{th} day.

    We have mentioned sentences in the Results of article; page 7, line 3-7: "Before the maturation period, the average body weight of triploid and diploid males was 16.6 and 10.7 g bigger than those of triploid and diploid females, respectively. Meanwhile, during maturation period, the average body weight of triploid and diploid males was 103.3 and 50.5 g bigger than those of triploid and diploid females, respectively."

17. Reviewer comment: Results; Where this came from now? You used AGR in Table 1 and didn't mentioned both in M&M. didn't get what do you mean… seems different than Fig 2. No..intersting of symbol

    Authors response: We have mentioned the formulas, include the specific growth rate (SGR) formula in the Materials and methods of article; pages 5 and 6, line 17-22 and line 1, respectively: "The formulas were used to calculate absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), respectively, as follows:.." and we have mentioned the symbol of SGR in the Figure 2 of article; page 24, line 3: "**Figure 2.** Schematic sequential specific growth rates (SGR) of triploid and diploid ..."

18. Reviewer comment: Results; Didn't get these numbers?? Too much confusing

    Authors response: As well as point 16, the dressing and edible carcass values obtained are the calculation in increasing percentages of dressing and edible carcass between triploid and diploid females and between triploid and diploid males, respectively.

We have mentioned sentences in the Results of article; pages 7 and 8, line 22-23 and line 1-2, respectively: "The dressing and edible carcass percentages of female triploid were 8.6 and 10.5% higher than those of female diploid, respectively. Meanwhile, the dressing and edible carcass percentages of male triploid were 2.1 and 5.9% higher than those of the diploids, respectively (Table 2)."

19. Reviewer comment: Table 1; Add "s" in group

    Authors response: We have revised and mentioned in the Table 1 of article; page 20: "Fish groups"

20. Reviewer comment: Figure 1; Please us same indicators for same groups in two graphs without grey background.

    Authors response: We have revised about indicator symbol in the Figure 1 of article; page 23.

For Reviewer 3

1. Reviewer comment: There is no statistical difference between the final weight of triploid females and the final weight of diploid females. The reason remains unexplained. According to the kinds of literature, triploidization lead to increase in sterility and growth. Energy for gamete production is reduced or inhibited. Gonadosomatic indices (GSI) were not nevertheless examined in this study. Therefore, it is not possible to comment on why triploid females have no superiority in terms of growth.

   Authors response: In this study showed that sex is crucial for growth in tilapia. In the Nile tilapia, sex dimorphism determine the speed of fish growth. It is proven that the influence of sex dimorphisms is more dominant than triploidy.

   Based on this study in Table 1 and Figures 1 and 2 show the body weight of female triploid was significantly difference compared to that of female diploid, while increasing body weight of female triploid was no significant difference than mixed-sex diploid because in the mixed-sex group, there are still male sex which has the potential for weight gain on average.

   On the other hand, we have also conducted studies on reproductive performances (gonadal morphology and histology) including gonadosomatic index (GSI) between triploid and diploid Nile tilapia, both male and female. Based on this study, GSI of female triploid has lowest than female diploid. We apologize, the reproductive performances data, including GSI is being submitted for review in other journals.

2. Reviewer comment: There is no homogeneity among groups in terms of initial weights and lengths. In the first two months after hatchings, triploid and diploid genders could reach equal weight by size-specific feeding regime, and then the study could be started without statistical difference among initial weights. There is a mistake in the methodology. As seen in Table 1, possible statistical differences among the initial mean weights of the groups were not analyzed, likewise among the final mean weights. This deficiency was wanted to be skipped because of heterogeneity.

   Authors response: We can not control and homogenize the initial weights and lengths. This is because since the first 2 months of previous rearing at laboratory scale, triploid tilapia has a grow faster and larger (body weight and total length) compared to diploid tilapia, although the triploid and diploid populations are from the same parents and are similar age. This treatment has been repeated 4 times though given the similar type and amount of feed, as we mentioned in the Maaterials and methods of article; page 4, line 7-12.

Therefore, we was conducted a mean sampling of the initial weight and length of the triploid and diploid fish, both male and female. We was used averages of initial weight and lenght among population. Even, we was used highest average of diploid among population obtained after rearing fry for 2 months at laboratory scale, before being used in this study (field scale). So that, the initial weights and lenghts that we use is a fact that occurs due to the difference in the triploidization treatment in Nile tilapia. We can not control to be homogenous because it might not be homogeneous from the start of the study. We only ensure that we use fish populations of the same age, both triploid and diploid.

3. Reviewer comment: What did cause the differences between survival rates of triploid and diploid? What is the advantage of triploidy in terms of survival in Tilapia?

   Authors response: In this study, survival rate (SR) is likely due to stressed fish after sampling, especially in female diploid which have a high likelihood of maturation, so fish are very susceptible to stress. This may be one of our weaknesses in controlling stress levels and survival of fish after sampling.

4. Reviewer comment: Selection of gender in Tilapia can be easily done by manual technique around 20-30 gr weights. So, any fish farmers would not like to culture tilapia with mixing two genders. If triploid female Tilapia are sterile which cause more somatic growth, mix culture of genders are logical and feasible. But, there are no results about sterility and GSI of triploid female, and also whether they reproduce or not in the mixed group according to this study.

   Authors response: Every month, we always do a total sampling of fish and observe sex through genetalia. During the sampling until the end of this study cleary matches the sex-grouped treatment. During the study, we did not find any all-female or all-male triploids reproducing, including mixed-sex triploid group. Except in diploid tilapia, both all-female and all-male groups are found to undergo maturity and reproduction, including mixed-sex group. This is also as we have mentioned the sentences in the Discussion of article; page 10, line 6-7.

   This is also supported by the observation of GSI and sterility of triploid tilapia, both males and females at the time of monthly sampling, the 3rd month until the 6th month, as the our other studies on the reproductive performances of triploid and diploid Nile tilapia, including GSI and sterility of those male and female fish are being submitted for review in other journals.

5. Reviewer comment: Abstract; gonadosomatic index is one of the most important criterion in triploidy studies of aquaculture. it should had been also investigated.

   Authors response: In the other studies, we were also investigated on reproduction performances, include GSI and sterility of triploid and diploid Nile tilapia, both male and female. However, these parameters would be submitted for review in the process of the other journals.

6. Reviewer comment: Materials and methods; Change “embryos” to “eggs”.

   Authors response: We have changed and mentioned word in the Materials and methods of article; page 3, line 24: “Artificially fertilized eggs ...”

7. Reviewer comment: Materials and methods; Change “closed water recirculation” to “recirculatimg”.

   Authors response: We have changed and mentioned word in the Materials and methods of article; page 4, line 5: “...a recirculating system ...”

8. Reviewer comment: Materials and methods; which weights? in sentences “Sexing was conducted...”

   Authors response: We have mentioned word in the Materials and methods of article; page 4, line 13-14: “... on the average fish weight of 6.5-10 g ...”

9. Reviewer comment: Materials and methods; Delete ”anus, urethra, and”

   Authors response: We have deleted word and mentioned in the Materials and methods of article; page 4, line 13: “.. observing the genital openings ..”.

10. Reviewer comment: Materials and methods; Change “During the first month of the rearing period, the” to “Firstly”.

    Authors response: We have changed and mentioned word in the Materials and methods of article; page 5, line 3: “Firstly, fish were fed ...”

11. Reviewer comment: Materials and methods; Add ad libitum and for 30 days and changed “while the fish” to “then they”.

    Authors response: We have mentioned word in the Materials and methods of article; page 5, line 4: “Firstly, fish were fed ... at satiation for 30 days, then they were fed ...”

    While, we continue to use the term at satiation because in this study, we fed in the fish litle by litle until the fish stop eating (not continuously), so we considered this as feed satiation and not ad libitum. Ad libitum assumed that feed is available at all times continuously.

12. Reviewer comment: Materials and methods; Change “at satiation” to “ad libitum”.

    Authors response: In this study, we continue to use the term at satiation because we fed in the fish litle by litle until the fish stop eating (not continuously), so we considered this as feed satiation and not ad libitum. Ad libitum assumed that feed is available at all times continuously..

13. Reviewer comment: Materials and methods; Change “collected” to “measured”.

    Authors response: We have changed and mentioned word in the Materials and methods of article; page 5, line 8: “... data were measured every month,...”

14. Reviewer comment: Results; Should be checked in terms of english grammar in the sentences “Based on the average body weight before the maturation period, triploid and diploid males had 16.6 and 10.7 g higher than females, respectively. Meanwhile, during the maturation period, triploid and diploid males had 103.3 and 50.5 g bigger than females, respectively.”

    Authors response: We have revised and mentioned the sentences in the Results of article; page 7, line 3-7: “Before the maturation period, the average body weight of triploid and diploid males was 16.6 and 10.7 g bigger than those of triploid and diploid females, respectively. Meanwhile, during maturation period, the average body weight of triploid and diploid males was 103.3 and 50.5 g bigger than those of triploid and diploid females, respectively."

15. Reviewer comment: Results; Times should be rewritten en d of the sentences as I stated above in the sentences "At 90 days old, all-female and mixed-sex triploids showed the same growth rate. At 120 to 180 days old, the mixed-sex triploid had higher specific growth rate (SGR), while at 120 days old all-female triploid had same SGR as all-male diploid. At 150 days old, all-female triploid, all-male and mixed-sex diploids had same SGR. At 180 days old, all-female triploid had the same SGR as the mixed-sex diploid."

    Authors response: We have revised and mentioned the sentences in the Results of article; page 7, line 11-17: "All-female and mixed-sex triploids groups showed the similar growth rate at the 90th day (Figure 2). The mixed-sex triploid group has higher specific growth rate (SGR) than other sex groups at 120th to 180th day, while all-female triploid group has similar SGR as all-male diploid group at the 120th day. On the other hand, all-female triploid and all-male and mixed-sex diploids groups have similar SGR at the 150th day. Meanwhile, all-female triploid group has similar SGR as the mixed-sex diploid group at the 180th day (Figure 2)."

16. Reviewer comment: Discussion; Delete "during 110 days grow-out period", "during 8 weeks", "during 175 days", and "during 12 weeks".

    Authors response: We have deleted word in the Discussion of article; page 8, line 17-18.

17. Reviewer comment: Discussion; Add "respectively" after "faster and lower"

    Authors response: We have mentioned the word in the Discussion of article; page 9, line 18: "... faster and lower, respectively than ..."

18. Reviewer comment: Discussion; Change "at 120 to 150 days old" to "at 120 to 150th day" and change "at 180 days old" to "at 180th day".

    Authors response: We have mentioned the word in the Discussion of article; page 9, line 21: "...groups at 120th to 150th day." and page 9, line 24: "... diploid at the 180th day (Figure 2)."

19. Reviewer comment: Discussion; "So, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth." To say this, gonadosomatic index should had been investigated.

    Authors response: In the other studies, we were also investigated on reproduction performances, include GSI of triploid and diploid Nile tilapia, both male and female. However, these parameters would be submitted for review in the process of the other journals.

20. Reviewer comment: Discussion; "The results of this study indicated that higher flesh percentages of female triploid compared to male triploid was because the female was more sterile than male, while the higher flesh percentages in triploid compared to diploid seemed correlated with normal in diploid and reducing in triploid through gonadal developments." Not understood. what you mean? how do you know females are more sterile than males? which method did you use for reaching this kind of result?

    Authors response: In the other studies, we was investigated reproduction performances, include gonadal morphological and histologycal of triploid and diploid Nile tilapia, both male and female, including sterility of fish. The study show that the sterility of triploid tilapia, both males and females at the time of monthly sampling, the 3rd month until the 6th month are the fact and clearly. The sterility data, as the our other studies on the reproductive performances of triploid and diploid Nile tilapia are being submitted for review in other journals.

21. Reviewer comment: References; correction of italic “Cyprinus carpio”.

    Authors response: We have revised and mentioned in the References of article; page 19, line 7: “... common carp (*Cyprinus carpio*) in ...”

Thus authors responses on comments, corrections, and suggestions of reviewers, we expect the reviewers and editor were pleased and understand it and we hope that this article will be corrected further. Thank you very much.

Best regards,

Akhmad Taufiq Mukti

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Selasa, 3 Desember 2019 15.22 GMT+7

Dear AKHMAD MUKTI

Even though you were asked before to revise your manuscript in terms of some deficiencies, it has been evaluated and has been found to be lacking in the respects stated below. You are requested to correct these deficiencies and re-submit your manuscript within 30 days so that its processing may begin immediately.

Deficiencies:

1. Other reasons (Look at technical comments)

Technical Comments: Until your manuscript complies with all of the journal's rules it will not be processed further.
--- You must write authors ORCid's to the cover page. (for all authors). You can visit https://orcid.org/ to get your unique ORCID IDs. (ODANG CARMAN ?)

We thank you for your interest in our journal.

Yours sincerely,

Article Title: Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (Oreochromis niloticus)
Article Code Number: VET-1905-79
Web address: http://online.journals.tubitak.gov.tr

YÜCEL UYAR
Journal Administrator

NOTE: To resubmit your manuscript, log onto the online system as corresponding author and find your manuscript in the 'Manuscripts Sent Back to Author Because of Deficiencies' list. Then you can resubmit your manuscript. Please do not use 'Submit New Manuscript' link for resubmission.

TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES, VET-1905-79

Dari: bmys-info@ulak.tubitak.gov.tr

Kepada: atm_mlg@yahoo.com

Tanggal: Selasa, 28 Januari 2020 20.46 GMT+7

Dear AKHMAD MUKTI,

We are pleased to inform you that your manuscript submitted to the TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES has been accepted for publication.

Yours sincerely,

MEHMET BAŞALAN
Editor-in-Chief

Manuscript Title: Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (Oreochromis niloticus)

Decision Report: Authors took into consideration the suggestions of referees and editor and completed the necessary corrections.

COPYRIGHT RELEASE FORM

TÜBİTAK - The Scientific and Technological Research Council of Turkey

Akademik Dergiler Müdürlüğü, Akay Caddesi No: 6, Bakanlıklar, 06420 Ankara, Turkey

Journal title: Turkish Journal of Veterinary and Animal Sciences

Manuscript title: **Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)**

Full names of all authors (in order to appear on manuscript): Akhmad Taufiq MUKTI, Odang CARMAN, Alimuddin ALIMUDDIN, Muhammad ZAIRIN JR., Muhammad Agus SUPRAYUDI

Name, address etc. of corresponding author: Akhmad Taufiq MUKTI, Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Unair, Jl. Mulyorejo Surabaya 60115, Indonesia. Tel.: +62 315911451; E-mail: atm_mlg@yahoo.com

ID Number: B 4869767

Telephone: +62 315911451

E-mail: atm_mlg@yahoo.com

Mobile phone: +62 81555637985

Turkish authors must supply their **ID card number**; foreign authors must supply their passport number (or, if not available, driving license number, ID card number, etc.).

On behalf of all authors, as the corresponding author of the manuscript, I warrant that

a) the manuscript submitted is my/our own original work;
b) all authors participated in the work in a substantive way and are prepared to take public responsibility for the work;
c) I was authorised by all authors to transfer all royalties related with the manuscript and to enter into a binding contract with TÜBİTAK as detailed in this Copyright Release Form, and I will be responsible in the event of all disputes that have occurred and that may occur,
d) all authors have seen and approved the manuscript as submitted;
e) e-mail and street addresses of all authors have been entered into the TÜBİTAK Academic Journals Manuscript Submission and Evaluation System correctly,
f) the manuscript has not been published and is not being submitted or considered for publication elsewhere;
g) the text, illustrations, and any other materials included in the manuscript do not infringe upon any existing copyright or other rights of anyone.
h) I transfer all financial rights, especially processing, reproduction, representation, printing, distribution, and online transmittal, to TÜBİTAK with no limitation whatsoever,

Notwithstanding the above, the Contributor(s) or, if applicable the Contributor's Employer, retain(s) all proprietary rights other than copyright, such as

a) patent rights;
b) to use, free of charge, all parts of this article for the author's future works in books, lectures, classroom teaching, or oral presentations;
c) the right to reproduce the article for their own purposes provided the copies are not offered for sale.

However, reproduction, posting, transmission or other distribution or use of the article or any material contained therein, in any medium as permitted hereunder, requires a citation to the Journal and appropriate credit to TÜBİTAK as publisher, suitable in form and content as follows: Title of article, author(s), journal title and volume/issue, Copyright© year.

As the corresponding author, I also warrant that "TÜBİTAK and the Journal Editors" will not be held liable against all copyright claims of any third party or in lawsuits that may be filed in the future, and that I will be the only person who will be liable in such cases. I also warrant that the article contains no libelous or unlawful statements, I/we did not use any unlawful method or material during the research, I/we obtained all legal permissions pertaining to the research, and I/we adhered to ethical principles during the research.

Corresponding Author's Full Name: Akhmad Taufiq MUKTI

Signature: 

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44:
© TÜBİTAK
doi:10.3906/vet-1905-79

Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)

Akhmad Taufiq MUKTI[1,*], Odang CARMAN[2], Alimuddin ALIMUDDIN[2], Muhammad ZAIRIN JR.[2], Muhammad Agus SUPRAYUDI[2]

[1]Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia

[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University (IPB), Bogor, Indonesia

Received: 24.05.2019 • **Accepted/Published Online:** 28.01.2020 • **Final Version:** 00.00.2020

Abstract: This study aimed to compare the growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia. The triploid population was obtained through heat shock at 41 °C for 4 min, 4 min after fertilization. Before sexing, 50 fish were reared in aquaria at a density of 1 fish L^{-1} for 2 months. After sexing, both triploid and diploid fish were grouped into all-male, all-female, and mixed-sex groups and reared in hapas at a density of 10 fish m^{-2} for 4 months. Each group was replicated three times. The highest body weight, body length, and growth rate were observed in all-male triploids, while the lowest of those parameters were obtained in all-female diploids. The highest survival rate was achieved in both all-male and mixed-sex triploids, and it did not significantly differ from the mixed-sex diploid ($P > 0.05$). The triploid fish had a higher edible carcass percentage than diploids. Proximate analysis indicated that the crude protein content of triploids was higher than that of diploids, while the crude lipid and ash contents were lower than those of diploids ($P < 0.05$). Triploid Nile tilapia had the best growth performances, including flesh quantity and quality, compared to diploids.

Key words: Growth performance, triploid production, monosex, mixed-sex, Nile tilapia

1. Introduction

Sterile fish are beneficial in aquaculture as the fish will reduce or even prevent the use of energy for reproduction in sterile metabolism processes. As a result, most of the anabolic energy will be transferred to somatic growth. Sterile fish also have the potential for a better survival rate compared to diploid fish. Devlin et al. [1] stated that the increase in the growth of fish brings substantial benefits in shortening culture period, improving the efficiency of feed utilization and the efficiency of production, and ensuring product availability. Correspondingly, culturing sterile fish is one of the best farming management approaches in aquaculture practices, as it enables the use of the metabolism pathway to obtain somatic tissue quickly instead of producing either sperm or eggs in the spawning season [2].

The high ability (uncontrolled) of tilapia reproduction causes unexpected density in the pond with varied size and slow growth, making it less commercially profitable in aquaculture. Sterilization is the best possible solution to solve the problems in tilapia culture [3]. Lutz [4] mentioned that among the future's aquaculture commodities, tilapia is a candidate fish to produce functionally sterile seeds on a large scale. The induction of triploidy is one of the methods of producing sterile fish. The culture of triploid fish could provide benefits such as increased growth, carcass production, survival rate, and flesh quality [5–7].

The production of triploid tilapia has been developed for more than four decades, and triploidy will be an effective management tool in tilapia farming in the future [8]. Triploid tilapia has small testes or ovaries, low gonad weight, and high body weight, protein utilization, and protein efficiency ratio compared to diploid tilapia. Thus, its farming is conceivably beneficial [9]. In some cases, the growth performances of triploid tilapia were reported to be superior or equal to those of diploid tilapia [10–12].

On the other hand, some studies indicated that male tilapia has faster growth compared to female tilapia [13–15]. The production level of monosex male tilapia farming was 10% higher compared to the mixed-sex population [16,17]. Associated with the presence of sexual dimorphism in terms of growth, many efforts were made to produce all-male seed populations for the purpose of monosex culture, which generally can be obtained through four common

* Correspondence: atm_mlg@yahoo.com

methods, namely manual sexing [18] at body size of 5–7 cm, hybridization [7,19], hormonal treatments [15,20–27], or chromosome set manipulations, such as androgenesis [18,28], to produce YY supermale parent stocks [29–31].

So far, the combined effects of triploidy and growth-related sexual dimorphism superiorities in tilapia are still unknown. The strain of fish, including tilapia, also possibly influences growth performance during the culture period. Therefore, the present study tries to clarify the effect of those superiorities on growth, survival rate, flesh percentage, and proximate composition of Nile tilapia during the grow-out period.

2. Materials and methods

2.1. Experimental fish preparation

In this study, the fish used were of the Wanayasa strain of Nile tilapia known as NIRWANA, produced through a family selection program between genetic improvement for farmed tilapia and genetically enhanced tilapia in Indonesia. The broodstocks were obtained from the Tilapia and Common Carp Aquaculture Development Agency in Purwakarta, West Java, Indonesia. Artificially fertilized eggs (4 min after insemination) were subjected to heat shock treatment at 41 °C for 4 min to produce triploid fish. This treatment produced triploid Nile tilapia of 91%–100%, as identified using the chromosome counting method according to Kligerman and Bloom [32] and Mukti et al. [33]. Embryos were incubated in glass funnels in a recirculating system, and diploid fish were produced using a similar procedure.

Larvae of both triploid and diploid were separately reared in 50-L aquaria at a density of 1 fish L^{-1}. A total of 10 aquaria were used for triploid and diploid fish, respectively. The 2-day-old fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Following, fish were transferred into 180-L aquaria, reared at a density of 4 fish L^{-1}, and fed on a commercial diet (40% crude protein content) for 30 days. Sexing was conducted morphologically by observing the genital openings at the average fish weight of 6.5–10 g to separate males and females of both triploid and diploid fish. The sexing was also confirmed by gonad preparation and observation using the squash method with acetocarmine stain. Twenty fish from different groups, namely all-male triploid, all-female triploid, mixed-sex triploid, all-male diploid, all-female diploid, and mixed-sex diploid, respectively, were prepared for performance evaluation.

2.2. Performances evaluation

Previously prepared all-male, all-female, and mixed-sex groups of both triploids and diploids were separately transferred and reared in floating nets of 2.0 m ′ 1.0 m ′ 0.7 m (mesh size of 10 mm) placed in concrete ponds of 20 m ′ 10 m ′ 1.5 m at a density of 10 fish m^{-2} with water exchange rate of 1 L s^{-1}. Water quality parameters, such as temperature, dissolved oxygen, and pH, were measured every week with ranges of 27–29 °C, 3.4–4.4 mg L^{-1}, and 6.7–7.3, respectively. Three floating nets were used as replication for each group. First, fish were fed on a 1-mm-diameter commercial diet (40% crude protein content) to satiation for 30 days, then they were fed on a 3-mm-diameter commercial diet (33% crude protein content) to satiation during the last 3 months (90 days), three times a day.

In general, the maturation period of tilapia begins for 90-day-old fish. In this study, the maturation period was also observed at the 90th day of fish rearing. The sex of the fish was checked monthly. Body weight (BW), body length (BL), mortality, and feed intake data were measured every month. Biomass gain; the relative percentages of biomass, BW, and BL gains of triploids compared to diploids; BW and BL gains; absolute growth rate (AGR); feed conversion ratio (FCR); and survival rate (SR) were analyzed based on data of initial and final grow-outs, while specific growth rate (SGR) was analyzed every month during 4 months of grow-out of fish. Dressing, edible carcass, and proximate data of male and female triploid and diploid fish were analyzed at the end of the experimental period.

The growth performances were calculated according to Hariati [34]. The formulas were used to calculate biomass gain (D), the relative percentage of triploid:diploid biomass gain, BW gain, the relative percentage of triploid:diploid BW gain, BL gain, the relative percentage of triploid:diploid BL gain, AGR, FCR, SR, and SGR, respectively, as follows:

$$\Delta \text{ Biomass (g)} = \text{Final biomass (g) - initial biomass (g)}$$

$$\Delta \text{ B 3N:2N (\%)} = \frac{\Delta \text{ biomass of triploid (g)} - \Delta \text{ biomass of diploid (g)}}{\Delta \text{ biomass of diploid (g)}} \times 100$$

$$\Delta \text{ BW (g)} = \text{Final body weight (g) - initial body weight (g)}$$

$$\Delta \text{ BW 3N:2N (\%)} = \frac{\Delta \text{ BW of triploid (g)} - \Delta \text{ BW of diploid (g)}}{\Delta \text{ BW of diploid (g)}} \times 100$$

$$\Delta \text{ BL (mm)} = \text{Final body length (mm) - initial body length (mm)}$$

$$\Delta \text{ BL 3N:2N (\%)} = \frac{\Delta \text{ BL of triploid (mm)} - \Delta \text{ BL of diploid (mm)}}{\Delta \text{ BL of diploid (mm)}} \times 100$$

$$\text{AGR (g day}^{-1}\text{)} = \frac{\text{Final body weight (g) - initial body weight (g)}}{\text{Length of rearing (days)}}$$

$$\text{FCR} = \frac{\text{Feed consumed by fish (g)}}{\Delta \text{ body weight of fish (g)}}$$

$$\text{SR (\%)} = \frac{\text{Live fish number at the final of rearing}}{\text{Live fish number at the initial of rearing}} \times 100$$

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\text{Ln final body weight - Ln initial body weight}}{\text{Length of rearing (days)}} \times 100$$

The dressing is the fish's body without head, fins, scales, and internal organs, while the edible carcass is a cut of the right and the left sides of the fish's body. The dressing and edible carcass data were determined according to Buchtova et al. [35] based on ten samples from males and females of both triploids and diploids, respectively. The dressing and the edible carcass percentages were calculated by the following formulas, respectively:

$$\text{Dressing (\%)} = \frac{\text{Dressing weight of fish}}{\text{Body weight of fish}} \times 100$$

$$\text{Edible carcass (\%)} = \frac{\text{Edible carcass weight of fish}}{\text{Body weight of fish}} \times 100$$

Increase of triploid dressing percentage (DP) and edible carcass percentage (ECP) compared to diploid was calculated using the relative percentages of triploid:diploid dressing and edible carcass formulas, respectively, as follows:

$$\Delta\text{Dressing 3N:2N (\%)} = \frac{\text{DP of triploid (\%) - DP of diploid (\%)}}{\text{DP of diploid (\%)}} \times 100$$

$$\Delta\text{Edible carcass3N:2N (\%)} = \frac{\text{ECP of triploid (\%) - ECP of diploid (\%)}}{\text{ECP of diploid (\%)}} \times 100$$

In addition, flesh proximate analysis of fish (crude protein, crude lipid, ash, and carbohydrate contents) was evaluated according to AOAC protocol [36] based on ten samples from both male and female triploids and diploids, respectively.

2.3. Statistical analysis

Data on growth performances (biomass gain, body weight and body length gains, AGR, and SGR); FCR, SR, and flesh percentages (dressing and edible carcass percentages); and proximate content were statistically analyzed using analysis of variance (ANOVA) with SPSS 10 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was followed by the ANOVA test with a confidence level of 95%.

3. Results

3.1. Growth performance, survival rate, and feed conversion ratio

The growth performances of the tested fish groups are shown in Table 1. The results showed that the growth of triploid fish was significantly higher ($P < 0.05$) compared to that of diploid. The biomass gains (D B 3N:2N) of

Table 1. The growth, survival rate, and feed conversion ratio performances of sex-grouped triploid and diploid Nile tilapia fish during 4-month grow-out period (n = 20).

Parameter	Fish groups					
	Triploid			Diploid		
	All-male	All-female	Mixed-sex	All-male	All-female	Mixed-sex
Initial biomass (g)	278.6 ± 5.2	190.0 ± 8.3	236.2 ± 6.0	205.0 ± 8.9	136.0 ± 8.8	183.4 ± 5.8
Final biomass (g)	8056.7 ± 405.5	5193.3 ± 445.6	7013.3 ± 551.4	6130.0 ± 366.6	4626.7 ± 277.6	5676.7 ± 465.0
DBiomass (g)	7778.1 ± 404.3^{a}	5003.0 ± 437.9^{e}	6777.1 ± 548.9^{b}	5925.0 ± 363.5^{c}	4490.7 ± 284.9^{f}	5493.2 ± 462.9^{d}
DB3N:2N (%)	31.3	11.4	23.4	-	-	-
Initial BW (g)	13.9 ± 0.3	9.5 ± 0.4	11.8 ± 0.3	10.3 ± 0.4	6.8 ± 0.4	9.2 ± 0.3
Final BW (g)	402.8 ± 20.3	278.5 ± 23.2	350.7 ± 27.6	317.0 ± 13.5	252.3 ± 10.2	288.3 ± 15.5
DBW (g)	388.9 ± 20.2^{a}	269.0 ± 22.8^{d}	338.9 ± 27.4^{b}	306.7 ± 13.6^{c}	245.5 ± 10.7^{e}	279.2 ± 15.3^{d}
DBW3N:2N (%)	26.8	9.6	21.4	-	-	-
Initial BL (mm)	99.2 ± 0.0	93.3 ± 0.0	92.5 ± 0.0	96.3 ± 0.0	92.8 ± 0.0	91.2 ± 0.0
Final BL (mm)	274.5 ± 2.1	241.3 ± 6.7	266.5 ± 5.6	250.0 ± 2.4	232.2 ± 1.9	243.4 ± 4.6
DBL (mm)	175.7 ± 2.1^{a}	147.9 ± 6.7^{d}	174.0 ± 5.6^{b}	153.7 ± 2.4^{c}	139.3 ± 1.9^{e}	152.2 ± 4.6^{c}
DBL3N:2N (%)	14.3	6.2	14.3	-	-	-
AGR (g day^{-1})	3.2 ± 0.2^{a}	2.2 ± 0.2^{d}	2.8 ± 0.2^{b}	2.6 ± 0.1^{c}	2.1 ± 0.1^{e}	2.3 ± 0.1^{d}
FCR	1.2 ± 0.1^{b}	1.4 ± 0.1^{c}	1.1 ± 0.0^{a}	1.2 ± 0.1^{b}	1.4 ± 0.0^{c}	1.4 ± 0.0^{c}
SR (%)	100.0 ± 0.0^{a}	93.3 ± 5.8^{c}	100.0 ± 0.0^{a}	96.7 ± 2.9^{b}	91.7 ± 2.9^{c}	98.3 ± 2.9^{ab}

D = Gain, D B 3N:2N = relative percentage of triploid:diploid biomass gain, BW = body weight, D BW 3N:2N = relative percentage of triploid:diploid body weight gain, BL = body length, D BL 3N:2N = relative percentage of triploid:diploid body length gain, AGR = absolute growth rate, FCR = feed conversion ratio, and SR = survival rate. Different superscripts in the same row indicate significant differences ($P < 0.05$).

all-male, all-female, and mixed-sex triploid fish were 31.3%, 11.4%, and 23.4% higher than those of diploids, respectively. A similar pattern was found in body weight gain (D BW 3N:2N) and body length gain (D BL 3N:2N). The highest values of body weight and length gains (26.8% and 14.3%, respectively) were observed in all-male triploids, followed by mixed-sex triploids (21.4% and 14.3%, respectively), while the lowest values (9.6% and 6.2%, respectively) were seen in all-female triploids. Furthermore, all-female diploid fish significantly showed the most inferior growth performance compared to other groups.

All-male triploids had the highest absolute growth rate (AGR) compared to other groups, followed by mixed-sex triploids, then all-male and all-female diploids. Meanwhile, the mixed-sex triploids had the best feed conversion ratio, followed by all-male triploids and diploids. The survival rates of all-male and mixed-sex triploids and mixed-sex diploids were higher compared to other groups, as shown in Table 1.

Figure 1 shows the monthly body weight and body length recorded during the 4-month grow-out period. In general, triploids grew faster than diploids, and all-male triploids showed the highest growth rate while all-female diploids showed the lowest growth rate.

In this study, it was observed that in both triploid and diploid fish, males grew faster than females during the experiment. In triploid and diploid groups, the biomass gains of the males were 55.5% and 31.9% higher than those of females, respectively. Before the maturation period, the average body weights of triploid and diploid males were 16.6 and 10.7 g greater than those of triploid and diploid females, respectively. Meanwhile, during the maturation period, the average body weights of triploid and diploid males were 103.3 and 50.5 g greater than those of triploid and diploid females, respectively. These results showed that the role of sexual dimorphism in the growth of Nile tilapia had a similar pattern as the role of ploidy level, the effects of which were highly significant during the maturation period.

All-female and mixed-sex triploid groups showed similar growth rates at the 90th day (Figure 2). The mixed-sex triploid group had a higher specific growth rate (SGR) than other sex groups at the 120th to 180th days, while the all-female triploid group had similar SGR as the all-male diploid group at the 120th day. On the other hand, all-female triploid and all-male and mixed-sex diploid groups had similar SGR at the 150th day. Meanwhile, the all-female triploid group had similar SGR as the mixed-sex diploid group at the 180th day (Figure 2).

3.2. Flesh percentage and proximate composition

The edible carcass percentages of male and female triploids were higher than those of diploids. The highest and lowest dressing percentages were found in triploid and diploid females, respectively ($P < 0.05$). The increase in dressing and edible carcass percentages of female triploids were 8.6% and 10.5% higher than those of female diploids, respectively. Meanwhile, the increase in dressing and edible carcass percentages of male triploids were 2.1% and 5.9% higher than those of the diploids, respectively (Table 2).

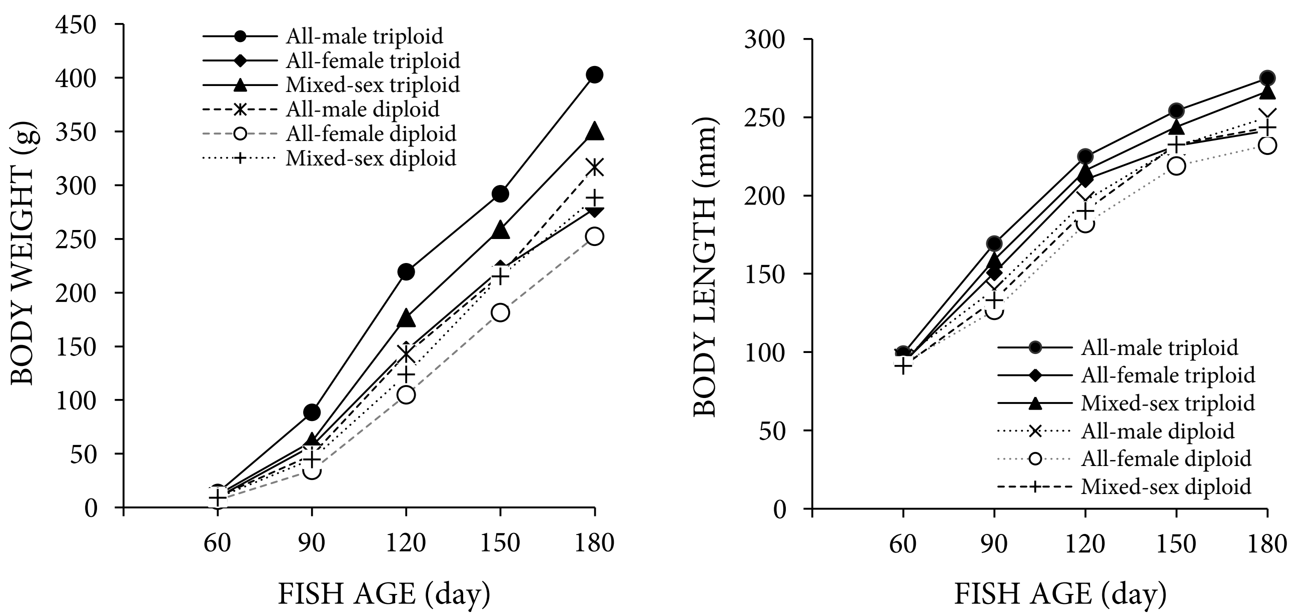

Figure 1. Body weight and body length of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4-month grow-out period.

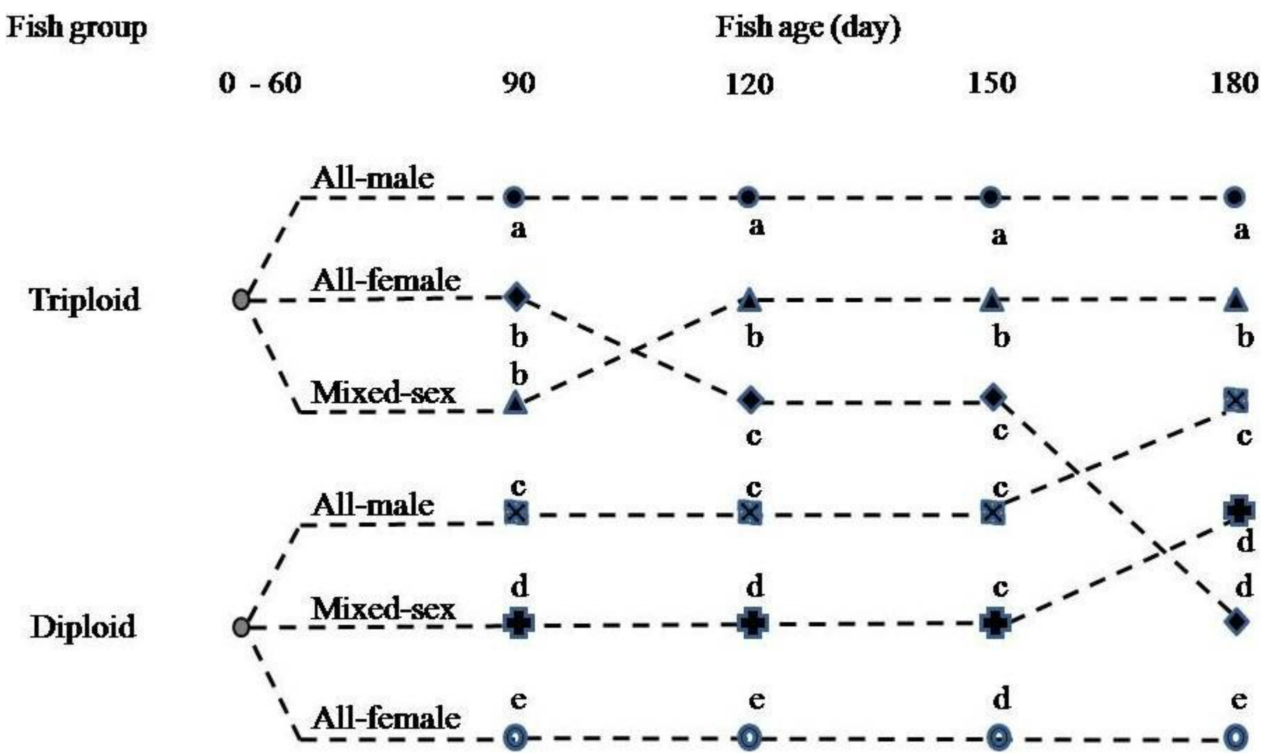

Figure 2. Schematic sequential specific growth rate (SGR) of triploid and diploid Nile tilapia fish during 4-month grow-out period. Different letters at the same fish age indicate significant differences (P < 0.05).

Table 2. Flesh percentages of male and female triploid and diploid Nile tilapia fish (n = 10).

Fish group		Body weight	Dressing		Edible carcass	
		(g)	Weight (g)	(%)	Weight (g)	(%)
Triploid	♂	414.1 ± 39.2^a	238.3 ± 19.9^a	57.6 ± 1.8^b	170.9 ± 16.0^a	41.3 ± 1.4^a
	♀	260.8 ± 24.0^c	154.0 ± 13.5^c	59.1 ± 1.6^a	109.4 ± 10.8^c	42.0 ± 1.2^a
Diploid	♂	332.0 ± 29.7^b	187.2 ± 18.4^b	56.4 ± 1.6^b	129.4 ± 12.4^b	39.0 ± 1.6^b
	♀	259.4 ± 14.1^c	141.0 ± 7.8^c	54.4 ± 1.3^c	98.5 ± 6.0^d	38.0 ± 1.4^b

Different superscripts in the same column indicate significant differences (P < 0.05).

Flesh proximate analysis of triploid and diploid fish is shown in Table 3. The crude protein content of female triploids was similar to that of male triploids; however, it was higher than that of diploid fish (P < 0.05). On the other hand, crude lipid and ash contents of male and female triploids were lower than those of diploids. There were no significant differences in carbohydrate contents between triploid and diploid fish.

4. Discussion

This study revealed that ploidy level and sexual dimorphism play essential roles in Nile tilapia growth performance. The high growth of male triploids and low growth of female diploids indicated that both ploidy level and sexual dimorphism significantly affected Nile tilapia growth (Table 1; Figures 1 and 2).

Tave [37] reported that triploidization leads to an increase in sterility and growth. The cell size of triploids is larger than that of diploids, and energy for gamete production is reduced or inhibited. In most cases, triploids showed heavier body size and faster growth than diploids in common carp (*Cyprinus carpio*) [38], African mud catfish (*Clarias gariepinus*) [39], Chinese catfish (*C. fuscus*) [40], and Atlantic salmon (*Salmo salar*) [41]. Besides, the performances of triploid fish were not only species- and age-dependent but also depended on the experimental

Table 3. Flesh proximate analysis of male and female triploid and diploid Nile tilapia fish (% dry weight) (n = 10).

Fish group		Crude protein	Crude lipids	Ash	Carbohydrates
Triploid	♂	85.6 ± 0.3^{ab}	5.1 ± 0.2^{b}	6.2 ± 0.2^{c}	3.2 ± 0.7^{a}
	♀	87.0 ± 1.1^{a}	5.0 ± 0.4^{b}	5.9 ± 0.0^{d}	2.2 ± 1.5^{a}
Diploid	♂	84.2 ± 1.3^{b}	5.9 ± 0.3^{a}	7.1 ± 0.0^{a}	2.8 ± 1.7^{a}
	♀	84.3 ± 1.8^{b}	5.5 ± 0.0^{a}	6.4 ± 0.3^{b}	3.8 ± 1.5^{a}

Different superscripts in the same column indicate significant differences (P < 0.05).

conditions and the interactions between the environment and genetics [7]. The individual body size of triploids was more significant due to the larger cell size compared to diploids [42]. However, Aliah et al. [43] reported that cell size was not correlated with organ size in sticklebacks (*Gasterosteus aculeatus*). Furthermore, in 2- to 3-month-old sunshine bass (*Morone* spp.), diploids grew faster compared to triploids [44].

The increase in triploid growth is due to the influence of sterility, diverting energy (nutrients) for somatic growth rather than gonadal development and sexual activity [14]. Most studies concluded that the significant difference in growth rate between triploid and diploid fish occurred during the maturation period in fish such as turbot (*Scophthalmus maximus*) [45] and European sea bass (*Dicentrarchus labrax*) [46]. In this study, it was found that the growth difference (30.0%) between triploid and diploid fish had already occurred before (≤90 days) and during the maturation period (90–180 days). Also, the growth of triploids showed more significant differences compared to diploids (39.3%). A similar phenomenon has been reported in fancy carp (*C. carpio*) [47].

The role of sexual dimorphism in growth in tilapia has been revealed in the last three decades. Male tilapia grew faster compared to females, so all-male monosex culturing in this species is worldwide applied. Similar cases were found in catfish (*C. gariepinus*) [48] and crucian carp (*Carassius auratus*) [49].

The comparison of the growth performance among the six groups showed that all-male triploid and all-female diploid fish grew faster and slower, respectively, than the fish in other groups during the experiment. The interaction effect between triploidy and sexual dimorphism in growth was not significant among all-female triploid, all-male diploid, and mixed-sex diploid groups at the 120th to 150th days. In the same groups, all-male diploids grew faster than the others and the interaction effect between triploidy and sexual dimorphism on growth was not significant among all-female triploids and mixed-sex diploids at the 180th day (Figure 2). This phenomenon seemed to be species-specific as found in rainbow trout (*Oncorhynchus mykiss*) by Tabata et al. [50], Mozambique tilapia (*O. mossambicus*) by Varadaraj and Pandian [51], and European sea bass by Felip et al. [52]. Those authors reported that female triploids grew faster than either male triploids, male and female diploids, and mixed-sex diploid.

The lowest growth was observed in all-female diploids, although it looked as if the female diploids went through rapid reproductive development and sexual maturity. Thus, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth. In this study, it was recorded that at the 120th day, the majority of female diploids began to spawn and incubate either fertilized or unfertilized eggs in the mouth. This generally allows the female to not feed during egg incubation for 15 days until the larvae can swim freely, as reported by Byamungu et al. [53]. In other words, the role of ploidy level in growth during the maturation period was significantly more important than that before the maturation period. These results also revealed that high body weight gain in male and female triploids during the maturation period seemed to be due to the sterility of triploid fish and the reproductive activity of diploid fish.

In this study, triploid fish had higher flesh percentages compared to diploids, and female triploids also had higher flesh percentages. Similar results were reported in gilthead sea bream (*Sparus aurata*) [54] and rainbow trout [55]. However, in common carp [56] up to the size of 400 g, the dressing weight of triploids was not significantly different from that of diploids. The results of this study indicated that female triploids had higher flesh percentages than male triploids as the females were more sterile than males, while the higher flesh percentages in triploids compared to diploids seemed correlated with normal gonadal development in diploids and reduced development in triploids.

Triploid Nile tilapia tends to be high in crude protein and low in crude lipid and ash compared to diploids. In terms of sex, both triploid and diploid male and female fish show the same crude protein, crude lipid, and carbohydrates contents, while the ash content is significantly different. This shows that triploidy in Nile tilapia affects flesh quality, especially crude lipid and ash contents. These results are supported by the findings of other researchers [5,6,11], but further studies are needed to gather more valuable information.

The interaction effect between triploidy and sexual dimorphism, strongly related to growth, had a positive contribution to production performance, especially during the maturation period. Based on the examination of various aspects related to production, the result revealed that all-male triploid Nile tilapia cultures have the potential to be developed. Hence, in the future, an applicable method for mass all-male triploid seed production should be considered. One of the possible strategic efforts is production of supermale tetraploids as parent stock by combining the chromosome set and hormonal manipulations.

Acknowledgments
This study was partially supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia through the BPP-DN scholarship program and a Post-Doctoral Research Grant. The authors would like to thank the deceased Prof. Komar Sumantadinata, who provided guidance and support during the study period, and the Head and staff of the Tilapia and Common Carp Aquaculture Development Agency at Purwakarta, West Java, Indonesia, for providing Nile tilapia (NIRWANA) broodstocks. The authors would also like to acknowledge the comments, corrections, and suggestions given by the reviewers, editor, and proofreaders to improve this article.

Conflict of Interest
The authors have no conflicts of interest to disclose.

References

1. Devlin RH, Biagi CA, Yesaki TY. Growth, viability and genetic characteristics of GH transgenic Coho salmon strains. Aquaculture 2004; 236 (1-4): 607-632. doi: 10.1016/j.aquaculture.2004.02.026
2. Galli L. Genetic Modification in Aquaculture: A Review of Potential Benefits and Risks. Canberra, Australia: Bureau of Rural Sciences; 2002.
3. Jayaprasad PP, Srijaya TC, Jose D, Papini A, Hassan A et al. Identification of diploid and triploid red tilapia by using erythrocyte indices. Caryologia 2011; 64 (4): 485-492. doi: 10.1080/00087114.2011.10589816
4. Lutz CG. Practical Genetics for Aquaculture. Fishing News Books, Oxford, UK: Blackwell Science; 2001.
5. Felip A, Zanuy S, Carrillo M, Piferrer F. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. Genetica 2001; 111 (1-3): 175-195.
6. Melamed P, Gong Z, Fletcher G, Hew CL. The potential impact of modern biotechnology on fish aquaculture. Aquaculture 2002; 204 (3-4): 255-269. doi: 10.1016/S0044-8486(01)00838-9
7. Dunham RA. Aquaculture and Fisheries Biotechnology: Genetic Approaches. Cambridge, UK: CABI Publishing; 2004.
8. Pradeep PJ, Srijaya TC, Bahuleyan A, Papini A. Can sterility through triploidy induction make an impact on tilapia industry? International Journal of Aquatic Science 2012; 3 (2): 89-96.
9. Pechsiri J, Yakupitiyage A. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 2005; 36 (1): 45-51. doi: 10.1111/j.1365-2109.2004.01182.x
10. Mol K, Byamungu N, Cuisset B, Yaron Z, Ofir M et al. Hormonal profile of growing male and female diploids and triploids of the blue tilapia (*Oreochromis aureus*)reared in intensive culture. Fish Physiology and Biochemistry 1994; 13 (3): 209-218. doi: 10.1007/BF00004359
11. Hussain MG, Rao GPS, Humayun NM, Randall CF, Penman DJ et al. Comparative performance of growth, biochemical composition, and endocrine profiles in diploid and triploid tilapia (*Oreochromis niloticus* L.). Aquaculture 1995; 138 (1-4): 87-97. doi: 10.1016/0044-8486(95)01079-3
12. Puckhaber B, Hörstgen-Schwark G. Growth and gonadal development of triploid tilapia (*Oreochromis niloticus*). In: ICLARM Conference Proceedings of the Third International Symposium on Tilapia in Aquaculture; Manila, Philippines; 1996. pp. 377-382.
13. Bhatta S, Iwai T, Miura T, Higuchi M, Maugars G et al. Differences between male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). Kathmandu University Journal of Science, Engineering and Technology 2012; 8 (II): 57-65. doi: 10.3126/kuset.v8i2.7326
14. Pradeep PJ, Srijaya TC, Papini A, Chatterji AK. Effects of triploidy induction on growth and masculinization of red tilapia [*Oreochromis mossambicus* (Peters, 1852) × *Oreochromis niloticus* (Linnaeus, 1758)]. Aquaculture 2012; 344-349: 181-187. doi: 10.1016/j.aquaculture.2012.03.006
15. Fuentes-Silva C, Soto-Zarazúa GM, Torres-Pacheco I, Flores-Rangel A. Male tilapia production techniques: a mini-review. African Journal of Biotechnology 2013; 12 (36): 5496-5502. doi: 10.5897/AJB11.4119
16. Dan NC, Little DC. The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in Northern Vietnam. Aquaculture 2000; 184 (3-4): 221-231. doi: 10.1016/S0044-8486(99)00329-4
17. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. Journal of Endocrinology 2001; 170 (1): 27-38. doi: 10.1677/joe.0.1700027

18. Cnaani A, Levavi-Sivan B. Sexual development in fish: practical applications for aquaculture. Sex Development 2009; 3 (2-3): 164-175. doi: 10.1159/000223080

19. Bartley DM, Rana K, Immink AJ. The use of interspecific hybrids in aquaculture and fisheries. Reviews in Fish Biology and Fisheries 2001; 10 (3): 325-337. doi: 10.1023/A:1016691725361

20. Popma TJ, Green BW. Sex Reversal of Tilapia in Earthen Ponds. Aquaculture Production Manual, Research and Development Series No. 35. Auburn, AL, USA: International Center for Aquaculture, Auburn University; 1991.

21. Pandian TJ, Sheela SG. Hormonal induction of sex reversal in fish. Aquaculture 1995; 138 (1-4): 1-22. doi: 10.1016/0044-8486(95)01075-0

22. Mukti AT. Optimation of 17α-methyltestosterone synthetic hormone dose and immersion duration in larvae on the success of Nile tilapia (*Oreochromis* sp.) sex reversal. BSc, Brawijaya University, Malang, Indonesia, 1998 (in Indonesian).

23. Romerio MP, Fencrich-Verani CSN, Santo De-Copmus BE, Pasilva AS. Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasileira de Zoologia 2000; 29 (3): 654-659. doi: 10.1590/S1516-35982000000300003

24. Mukti AT, Priyambodo B, Rustidja, Widodo MS. Optimization of both 17α-methyltestosterone synthetic hormone dosage and dipping duration of Nile tilapia (*Oreochromis* sp.) larvae on sex reversal efficacy. BIOSAIN Journal of Life Science 2002; 2 (1): 1-8 (in Indonesian with an abstract in English).

25. Mohamed AH, Traifalgar RFM, Serrano AE Jr, Peralta JP, Pedroso FL. Dietary administration of dehydroepiandrosterone hormone influences the sex differentiation of hybrid red Tilapia (*O. niloticus* × *O. mossambicus*) larvae. Journal of Fisheries and Aquatic Science 2012; 7 (6): 447-453. doi: 10.3923/jfas.2012.447.453

26. Beaven U, Muposhi V. Aspects of a monosex population of (*Oreochromis niloticus*) fingerling produced using 17-α methyltestosterone hormone. Journal of Aquaculture Research & Development 2012; 3 (3): 132. doi: 10.4172/2155-9546.1000132

27. Dagne A, Degefu F, Lakew A. Comparative growth performance of monosex and mixed-sex Nile tilapia (*Oreochromis niloticus* L.) in pond culture system at Sebeta, Ethiopian. International Journal of Aquaculture 2013; 3 (7): 30-34. doi: 10.5376/ija.2013.03.0007

28. Ezaz MT, Myers JM, Powell SF, McAndrew BJ, Penman DJ. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 2004; 232 (1-4): 205-214. doi: 10.1016/j.aquaculture.2003.08.001

29. Müller-Belecke A, Hörstgen-Schwark G. A YY-male (*Oreochromis niloticus*) strain developed from an exceptional mitotic gynogenetic male and growth performance testing of genetically all-male progenies. Aquaculture Research 2007; 38 (7): 773-775. doi: 10.1111/j.1365-2109.2007.01712.x

30. Aliah RS, Sumantadinata K, Maskur, Naim S. GESIT tilapia: Indonesia's genetic supermales. Global Aquaculture Advocate 2010; 3: 36-37.

31. Turra EM, Oliveira DAA, Teixeira EA, Luz RK, Prado SA et al. Reproduction control in Nile tilapia (*Oreochromis niloticus*) by sexual and chromosome set manipulation. Revista Brasileira de Reprodução Animal 2010; 34 (1): 21-28.

32. Kligerman AD, Bloom SE. Rapid chromosome preparation from solid tissues of fish. Journal of the Fisheries Research Board of Canada 1977; 34: 266-269. doi:10.1139/f77-039

33. Mukti AT, Carman O, Alimuddin, Muhammad Zairin Jr. A rapid chromosome preparation technique without metaphase arrest for ploidy determination in Nile tilapia (*Oreochromis niloticus*). Caryologia 2016; 69 (2): 175-180. doi: 10.1080/00087114.2016.1152112

34. Hariati AM. Fish Feed. Nuffic/Unibraw/Luw/Fish Fisheries Project. Malang, Indonesia: Brawijaya University; 1989 (in Indonesian).

35. Buchtova H, Svobodova Z, Kocour M, Velišek J. Evaluation of the dressing percentage of 3-year-old experimental scaly crossbreds of the common carp *Cyprinus carpio* (Linnaeus, 1758) in relation to sex. Acta Veterinaria Brno 2006; 75 (1): 123-132. doi: 10.2754/avb200675010123

36. AOAC. Official Methods of Analysis. 18th ed. Washington, DC, USA: Association of Official Analytical Chemists; 2005.

37. Tave D. Genetics for Fish Hatchery Managers. Westport, CT, USA: Avi Publishing; 1993.

38. Mukti AT, Rustidja, Sumitro SB, Djati MS. Polyploidization of common carp(*Cyprinus carpio* L.). BIOSAIN Journal of Life Science 2001; 1 (1): 111-123 (in Indonesian with an abstract in English).

39. Lawson EO, Ishola HA. Effects of cold shock treatment on the survival of fertilized eggs and growth performance of the larvae of African mud catfish *Clarias gariepinus* (Burchell, 1822). Research Journal of Fisheries and Hydrobiology 2010; 5 (2): 85-91.

40. Qin JG, Fast AW, Ako H. Grow-out performance of diploid and triploid Chinese catfish (*Clarias fuscus*). Aquaculture 1998; 166 (3-4): 247-258. doi: 10.1016/S0044-8486(98)00287-7

41. Burke HA, Sacobie CFD, Lall SP, Benfey TJ. The effect of triploidy on juvenile Atlantic salmon (*Salmo salar*) response to varying levels of dietary phosphorus. Aquaculture 2010; 306 (1-4): 295-301. doi: 10.1016/j.aquaculture.2010.05.002

42. Piferrer F, Beaumont A, Falguière JC, Flajšhans M, Haffray P et al. Polyploid fish and shellfish: production, biology, and applications to aquaculture for performance improvement and genetic containment. Aquaculture 2009; 293 (3-4): 125-156. doi: 10.1016/j.aquaculture.2009.04.036

43. Aliah RS, Yamaoka K, Inada Y, Taniguchi N. Effects of triploidy on tissue structure of some organs in ayu. Nippon Suisan Gakkaishi 1990; 56 (4): 569-575. doi: 10.2331/suisan.56.569

44. Kerby JH, Everson JM, Harrell RM, Geiger JG, Starling CC et al. Performance comparisons between diploid and triploid sunshine bass in freshwater ponds. Aquaculture 2002; 211 (1-4): 91-108. doi: 10.1016/S0044-8486(02)00009-1

45. Cal RM, Vidal S, Gómez C, Álvarez-Blázquez B, Martínez P et al. Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). Aquaculture 2006; 251 (1): 99-108. doi:10.1016/j.aquaculture.2005.05.010

46. Felip A, Piferrer F, Zanuy S, Carrillo M. Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons. Journal of Fish Biology 2001; 58 (1): 76-88. doi: 10.1111/j.1095-8649.2001.tb00500.x

47. Taniguchi N, Kijima A, Tamura T, Takegami K, Yamasaki I. Color, growth, and maturation in ploidy-manipulated fancy carp. Aquaculture 1986; 57 (1-4): 321-328. doi: 10.1016/0044-8486(86)90210-3

48. Achegbulu CE, Okonji VA, Obi A. Growth and economic performance of diploid and triploid African catfish (*Clarias gariepinus*) in outdoor concrete tanks. International Journal of Genetics 2013; 3 (1): 1-6. doi: 10.5829/idosi.ijg.2013.3.1.738

49. Chen S, Wang J, Liu SJ, Qin QB, Xiao J et al. Biological characteristics of an improved triploid crucian carp. Science in China Series C: Life Sciences 2009; 52 (8): 733-738. doi: 10.1007/s11427-009-0079-3

50. Tabata YA, Rigolino MG, Tsukamoto RY. Production of all-female triploid rainbow trout (*Oncorhynchus mykiss*) [Pisces, Salmonidae]. III. Growth up to first sexual maturation. Boletim do Instituto de Pesca 1999; 25: 67-76.

51. Varadaraj K, Pandian TJ. Production of all-female sterile-triploid (*Oreochromis mossambicus*). Aquaculture 1990; 84 (2): 117-123. doi: 10.1016/0044-8486(90)90342-K

52. Felip A, Carrillo M, Zanuy S. Older triploid fish retain impaired reproductive endocrinology in the European sea bass (*Dicentrarchus labrax*). Journal of Fish Biology 2009; 75 (10): 2657-2669. doi: 10.1111/j.1095-8649.2009.02458.x

53. Byamungu N, Darras VM, Kühn ER. Growth of heat-shock induced triploids of blue tilapia (*Oreochromis aureus*) reared in tanks and in ponds in Eastern Congo: feeding regimes and compensatory growth response of triploid females. Aquaculture 2001; 198 (1-2): 109-122. doi: 10.1016/S0044-8486(00)00605-0

54. Haffray P, Bruant JS, Facqueur JM, Fostier A. Gonad development, growth, survival and quality traits in triploids of the protandrous hermaphrodite gilthead sea bream (*Sparus aurata* L.). Aquaculture 2005; 247 (1-4): 107-117. doi: 10.1016/j.aquaculture.2005.02.037

55. Werner C, Poontawee K, Mueller-Belecke A, Horstgen-Schwark G, Wicke M. Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. Archiv Tierzucht 2008; 51 (1): 71-83. doi: 10.5194/aab-51-71-2008

56. Basavaraju Y, Mair GC, Kumar HMM, Kumar SP, Keshavappa GY et al. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp (*Cyprinus carpio*) in Karnataka, India. Aquaculture 2002; 204 (3-4): 407-418. doi: 10.1016/S0044-8486(01)00827-4